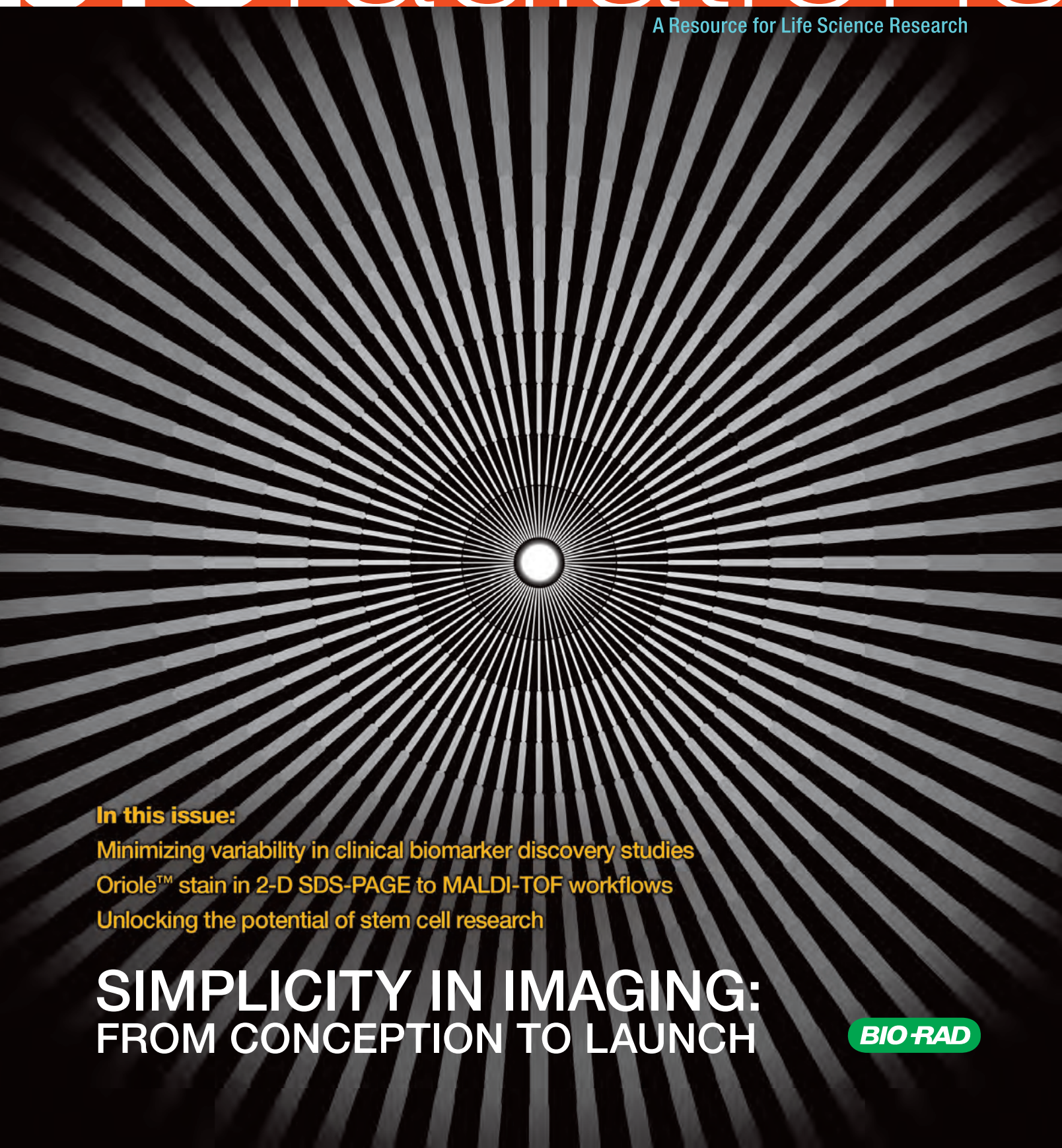
131

bioradiations

A Resource for Life Science Research



In this issue:

Minimizing variability in clinical biomarker discovery studies

Oriole™ stain in 2-D SDS-PAGE to MALDI-TOF workflows

Unlocking the potential of stem cell research

SIMPLICITY IN IMAGING:
FROM CONCEPTION TO LAUNCH

"Imageffortlessly in seconds. Now that's what I'm talking about."

Results at the push of a button.

Save time, space, and hassle with the new Gel Doc™ EZ imaging system. As the name implies, it's all about making life in the lab easier — giving you high quality images and analysis with ease and accuracy.

Simply slide in a tray, press the green button, and you'll have publication-ready images in seconds.

The new Gel Doc EZ imager is designed to meet the gel documentation needs of a busy lab.

- Compact design frees up bench space
- No need for manual control of filters, lenses, or lights
- Superior image quality makes images smooth and appealing at any zoom level
- Modular design means an upgrade path that is simple and budget friendly

See just how simple it is to imageffortlessly. Visit **www.bio-rad.com/ad/GelDocEZ** or contact your Bio-Rad sales representative to learn more.

Research. Together.

bioradiations

BIO-RAD REGIONAL OFFICE TELEPHONE NUMBERS

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Austria 43-1-877-89-01
Belgium 32-9-385-55-11
Brazil 55-31-3689-6600
Canada 905-364-3435
China 86-21-6426-0808
Czech Republic 420-241-430-532
Denmark 45-44-52-10-00
Finland 358-09-804-22-00
France 33-1-47-95-69-65
Germany 49-89-31884-0
Greece 30-210-777-4396
Hong Kong 852-2-789-3300
Hungary 36-1-459-6100
India 91-124-402-9300
Israel 03-963-6050
Italy 39-02-216091
Japan 81-03-6361-7000
Korea 82-2-3473-4460
Mexico 52-555-488-7670
The Netherlands 31-318-540666
New Zealand 0508-805-500
Norway 47-23-38-41-30
Poland 48-22-331-99-99
Portugal 351-21-472-7700
Russia 7-495-721-14-04
Singapore 65-6415-3188
South Africa 27-861-246-723
Spain 34-91-590-5200
Sweden 46-8-555-12700
Switzerland 41-61-717-9555
Taiwan 88-62-2578-7189
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If you have comments or suggestions regarding *BioRadiations*, please email us at **bioradiations@bio-rad.com**.

TO OUR READERS

Accurate analysis of 2-D gel electrophoresis data relies on the ability to capture gel images with high sensitivity, clarity, and reproducibility. This step in the electrophoresis workflow, however, is often among the most challenging, because imaging systems that offer complexity in terms of analysis are often also complicated to operate, requiring users to manually set and adjust — then recall at a later date — settings with each image capture. Bio-Rad has invested years in moving imaging technology in a simpler direction — without sacrifice to functionality. The latest imaging system, the Gel Doc™ EZ imager, represents a culmination of these efforts, offering one-button operation for a variety of electrophoresis applications. The cover article traces the evolution of this ground-breaking imager, from conception to its development and validation, and offers insight into how this product is already making a difference in researcher laboratories.

COVER STORY

DEPARTMENTS

TECHNICAL REPORTS

TC10™ Automated Cell Counter

Researchers often need to count cells prior to cell culture or before starting processes and analytical techniques that require an accurate and consistent number of input cells. These processes include transfection, cell proliferation, viability studies, and quantitative PCR. Counting cells with a microscope and hemocytometer is a tedious task with varying results. The TC10 cell counter is truly automated, providing a total count of mammalian cells and a live/dead ratio in one simple step with accurate, reproducible results. Cell culture data are retrieved faster and easier than when using a hemocytometer.

With the TC10 automated cell counter, you can:

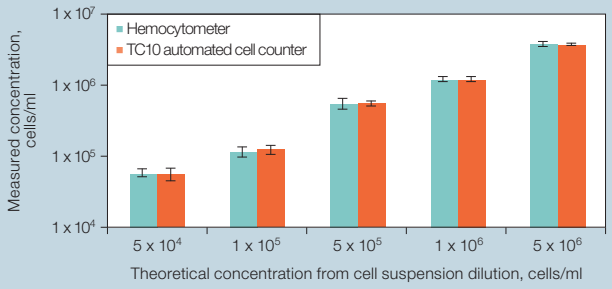
- **Fit cell counting into your schedule** — count cells quickly, accurately, and consistently within 30 sec using the built-in auto-focus
- **Configure results to your needs** — determine total cell count without dye, or use trypan blue dye to assess total cell count and cell viability
- **Have results at your fingertips** — print count results and dilution calculations from the TC10 thermal label printer
- **Easily archive your results** — transfer counts and cell images using a USB key and access up to 100 counts stored in the onboard memory
- **Trust your counts** — confirm instrument functionality with the TC10 verification slide
- **Conserve precious cells** — use only 10 µl of suspended cells

Accuracy is consistent and comparable to results obtained with a hemocytometer when counting cells in the range of 5 x 10^4–1 x 10^7 cells/ml and within 6–50 µm cell diameter. The broad concentration range eliminates the need to dilute cells prior to counting, which may be necessary when counting cells by other methods. The counting algorithm successfully discriminates and counts individual cells within clusters of up to five cells, providing accurate counts without the need to extensively declump cells prior to loading. The accurate, consistent cell counts from the TC10 counter lead to more reproducible results with downstream processes and analysis, saving money and time by enabling successful experiments the first time.

For more information, visit **www.bio-rad.com/TC10**, or request **bulletins 5929** and **5944**.

Key features include:

- **Cell viability** — the TC10 cell counter can count samples with or without trypan blue dye. Simply add the dye solution to the cell suspension, insert the slide, and the TC10 counter auto-detects the presence of dye in the sample — no user input is required. Along with the cell count, it assesses cell viability via trypan blue dye exclusion in just 30 sec
- **Multifocal plane analysis** — every cell is analyzed across multiple focal planes to determine whether it is live or dead. Using a single focal plane to assess cell viability can lead to undercounting of live cells because of light scattering and the alignment of cells at different heights in a counting chamber
- **Data storage** — for easy accessibility of data, results from 100 counts are stored in the TC10 cell counter and can be exported via the USB port and opened in a Microsoft Excel spreadsheet. Images of the cells can be viewed on the instrument; annotated JPEG files can be exported via the USB port. A histogram of approximate cell size distribution can be viewed after each count
- **TC10 counting slides** — the counter uses disposable TC10 counting slides with a patent-pending design that ensures even sample distribution of cells throughout the counting chamber for accurate, consistent cell counts



The TC10 cell counter demonstrates accurate cell counts across an extended range of cell concentrations. MEF cells were concentrated, serially diluted, and counted with a hemocytometer and a TC10 automated cell counter. The TC10 counter and hemocytometer cell counts showed no statistically significant differences. Precision is indicated by the standard deviations; error bars represent average standard deviations. Cell counts on the TC10 counter were performed on four different instruments with six sample replicates.

Specifications

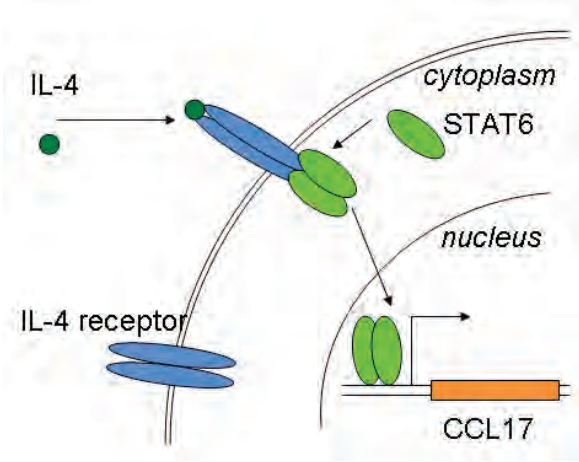
Counting time	30 sec
Cell concentration range	5 x 10^4–1 x 10^7 cells/ml
Cell diameter range	6–50 µm
Sample volume	10 µl
Data storage	100 counts
Data export	Via USB flash drive
Dimensions (W x D x H)	19 x 15 x 25.4 cm (7.5 x 6 x 10")
Weight	2.2 kg (4.8 lb) (without the external power supply)

Ordering Information

Catalog #	Description
145-0001	TC10 Automated Cell Counter, 100–240 V, includes instrument, USB key, 30 TC10 dual-chamber counting slides (60 counts), 1.5 ml TC10 trypan blue dye
145-0009	TC10 Automated Cell Counter with Printer, 100–240 V, includes instrument, USB key, TC10 thermal label printer, 1 roll of 185 labels, 30 TC10 dual-chamber counting slides (60 counts), 1.5 ml TC10 trypan blue dye
145-0003	TC10 Counting Kit, includes 30 TC10 dual-chamber counting slides (60 counts), 1.5 ml TC10 trypan blue dye

Video Release — Using an Automated Cell Counter to Simplify Gene Expression Studies

Using an Automated Cell Counter to Simplify Gene Expression Studies: siRNA Knockdown of IL-4 Dependent Gene Expression in Namalwa Cells



Overview of the IL-4 signaling pathway as seen in the automated cell counter video.

For additional details, request **bulletin 5860**.

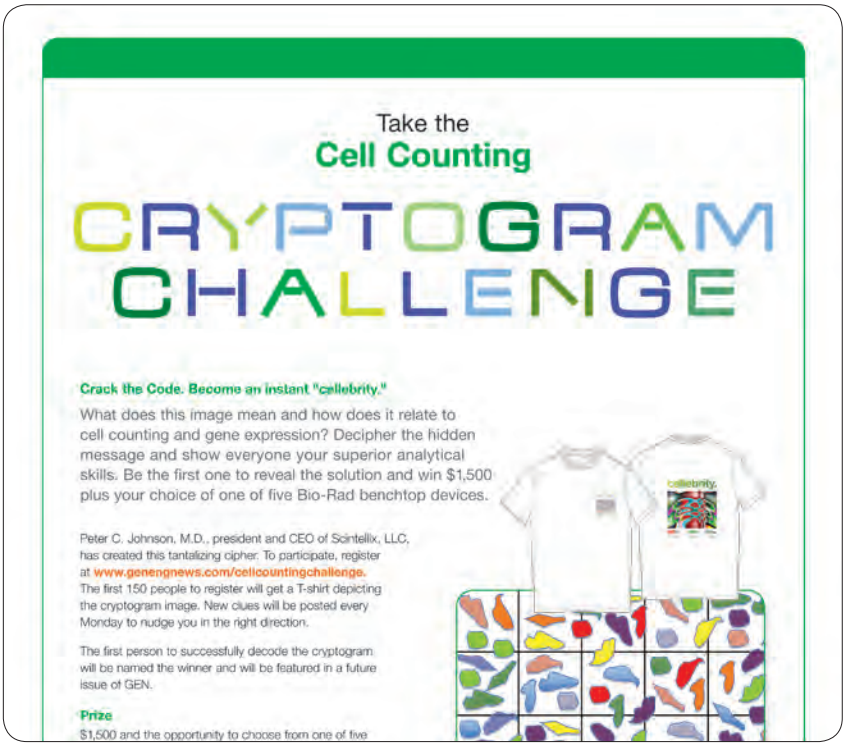
siRNA studies are conducted to study the effects of downregulating single genes as well as to interrogate signaling pathways and other complex interaction networks. These pathway analyses require the use of both relevant cellular models and methods that cause less perturbation to the cellular physiology.

Bio-Rad has released a video entitled "Using an Automated Cell Counter to Simplify Gene Expression Studies: siRNA Knockdown of IL-4 Dependent Gene Expression in Namalwa Cells," which investigates the role of the transcriptional activator STAT6 in IL-4 dependent gene expression of CCL17 in a Burkitt lymphoma (Namalwa) cell line. The video demonstrates multiple critical steps for successful siRNA experiments and the many ways to simplify the work while improving the data quality. Topics covered include how to perform a complete pathway study from collecting and counting the cells prior to electroporation through posttransfection real-time PCR gene expression analysis. The techniques demonstrated are useful for a wide range of siRNA-based experiments on both adherent and suspension cells.

The peer-reviewed video can be found at the following locations:

- **www.bio-rad.com/transfectiontips** — click the Educational Tools overlay
- **www.biorad-ads.com/video/09-0998-CellCounter**

Are You the Next "Cellebrity"?


Take the
Cell Counting
CRYPTOGRAM CHALLENGE

Crack the Code. Become an instant "cellebrity."

What does this image mean and how does it relate to cell counting and gene expression? Decipher the hidden message and show everyone your superior analytical skills. Be the first one to reveal the solution and win $1,500 plus your choice of one of five Bio-Rad benchtop devices.

Peter C. Johnson, M.D., president and CEO of Scintellix, LLC, has created this tantalizing cipher. To participate, register at www.genengnews.com/cellcountingchallenge. The first 150 people to register will get a T-shirt depicting the cryptogram image. New clues will be posted every Monday to nudge you in the right direction.

The first person to successfully decode the cryptogram will be named the winner and will be featured in a future issue of GEN.

Prize

$1,500 and the opportunity to choose from one of five

Crack the Code and Become an Instant Cellebrity

Bio-Rad has teamed up with GEN and Scintellix to bring you a series of cryptogram challenges in search of the winning cellebrity. Cryptograms are updated quarterly, with each new challenge providing a new level of complexity. Scientists from all over the world will challenge their pattern recognition and problem-solving skills as important data are revealed through weekly clues. Each quarter, a cellebrity will win $1,500 and a benchtop device (see prizes) from Bio-Rad. The first 150 people to register will receive a free T-shirt depicting the cryptogram image.

Prizes

The first to solve the contest will be awarded $1,500 and named the cellebrity. Additionally, this researcher will be given the opportunity to choose one of the following: Gene Pulser Xcell™ eukaryotic electroporation system, MyCycler™ personal thermal cycler, TC10™ automated cell counter, SmartSpec™ Plus spectrophotometer, or VersaFluor™ fluorometer.

Contest Duration

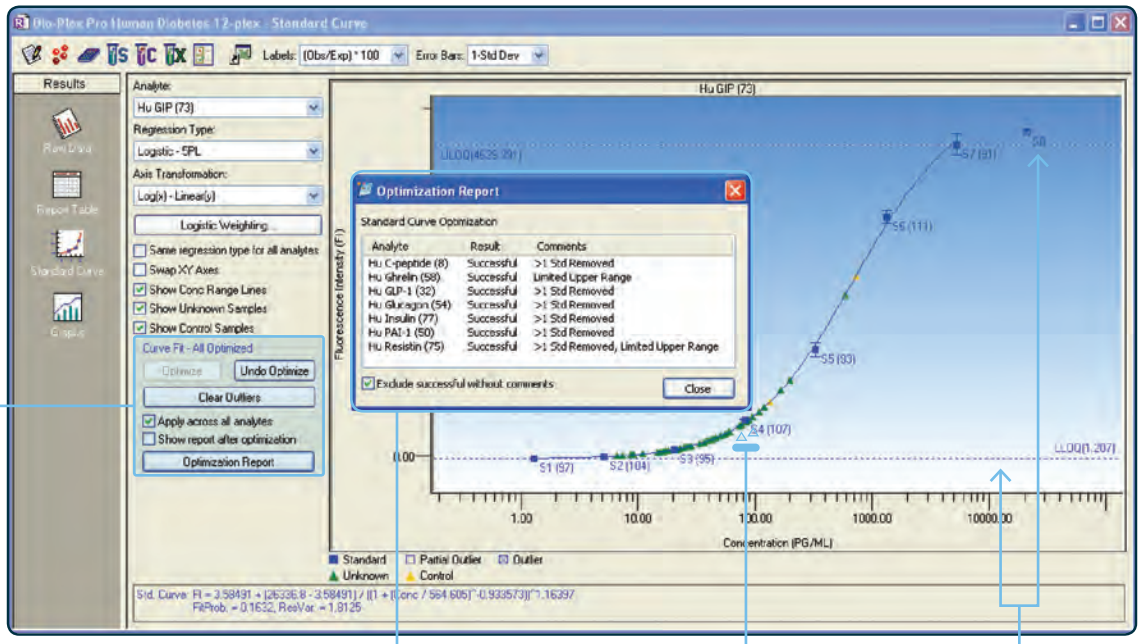
The contest is conducted quarterly throughout 2010, until the cipher has been decoded (for each quarter).

For full contest details, visit **www.bio-rad.com/ad/cellebrity**.

Latest Additions to the Bio-Plex® Multiplex Suspension Array System

Bio-Plex Manager™ Software Version 6.0

Bio-Plex Manager software version 6.0 is a comprehensive, all-in-one software package that provides system control, validation, calibration, data acquisition, and advanced data analysis for multiplex assays. New features of this latest version of Bio-Plex Manager software include simplified protocol editing, automated data processing, and the Gene Manager data analysis tool.



NEW! Identify the state of your data analysis session with dynamic on-screen feedback (this example indicates that all curves in the data file have been automatically optimized).

Review the optimization report to identify possible problem curves that you may want to review or try to improve manually. Example shows successful optimization with comments.

>1 Std Removed — More than one standard was removed in order to increase the usable range of the curve.

Limited Upper Range — 2 or more high concentration standards have poor recovery and therefore the upper range of your curve is not usable.

NEW!

Visualize unknowns and controls on your standard curve.
▲, unknowns;
▲, controls.

NEW!

Quickly identify usable range of each assay. Recommended Upper Limit of Quantitation and Lower Limit of Quantitation are identified on each graph.

NEW!

Bio-Plex Manager software automates data optimization — use version 6.0 and become an instant expert.

Ordering Information

Catalog #	Description
Desktop Version (for analysis only)	
171-STND01	Bio-Plex Manager 6.0 Desktop License, Standard Edition, Single User
Upgrades and Conversions	
171-SUPG30	Bio-Plex Manager 6.0 Upgrade for Bio-Plex Manager 3.0 (Workstation) Instrument Control Software
171-SUPG40	Bio-Plex Manager 6.0 Upgrade for Bio-Plex Manager 4.0 (Workstation) Instrument Control Software
171-SUPG41	Bio-Plex Manager 6.0 Upgrade for Bio-Plex Manager 4.1 (Workstation) Instrument Control Software
171-SUPG50	Bio-Plex Manager 6.0 Upgrade for Bio-Plex Manager 5.0 (Workstation) Instrument Control Software
171-STND23	Bio-Plex Manager 6.0 Software for IS 2.3 System

Bio-Plex Pro™ Human and Mouse Diabetes Assays

The Bio-Plex Pro diabetes assays offer enhanced:

- **Performance** — with a short 1 hr incubation time and expanded assay working ranges
- **Multiplexing** — combine with Bio-Plex Pro cytokine assays to understand the effects they play in angiogenesis, inflammation, and other metabolic diseases
- **Convenience** — All-in-one kit contains everything you need to start your studies
- **Flexibility** — choose only the singleplex assays you want to study

	NEW! Human Diabetes 10-Plex Assay	NEW! Mouse Diabetes 8-Plex Assay
Adiponectin*	•	•
Adipsin*	•	—
C-Peptide	•	—
Ghrelin	•	•
GIP	•	•
GLP-1	•	•
Glucagon	•	•
Insulin	•	•
Leptin	•	•
PAI-1	•	•
Resistin	•	•
Visfatin	•	—

*Due to different dilution schemes, adiponectin and adipsin are offered in singleplex and 2-plex formats only.

For more information on the human diabetes panel, request **bulletin 5966**.

For more information on the mouse diabetes panel, request **bulletin 5943**.

For more information on multiplexing diabetes and cytokine assays, request **bulletin 5975**.

The Bio-Plex 3D Suspension Array System

Accelerate your research with the Bio-Plex 3D suspension array system. Expanded multiplexing capability, faster time to results, and automation capability make it the platform of choice for high-throughput testing for nucleic acid and protein applications.

Key features include:

- <20 min read time
- 96- and 384-well capability
- Measure up to 500 analytes in a single sample
- LIS/LIMS database and robotics interfacing
- Bio-Plex Manager 6.0 software for analysis

Obtain the same quality results you get on a Bio-Plex 200 system in half the time.

Bio-Plex 3D suspension array system includes Bio-Plex Manager software 6.0.

For more information on the Bio-Plex 3D system, visit **www.bio-rad.com/Bio-Plex3D**, or request **bulletin 5967**.

Bio-Plex Pro Magnetic COOH Beads

Create unique protein and nucleic acid Bio-Plex assays using the Bio-Plex Pro magnetic COOH beads and the amine coupling kit. Twenty regions (1 ml) are available.

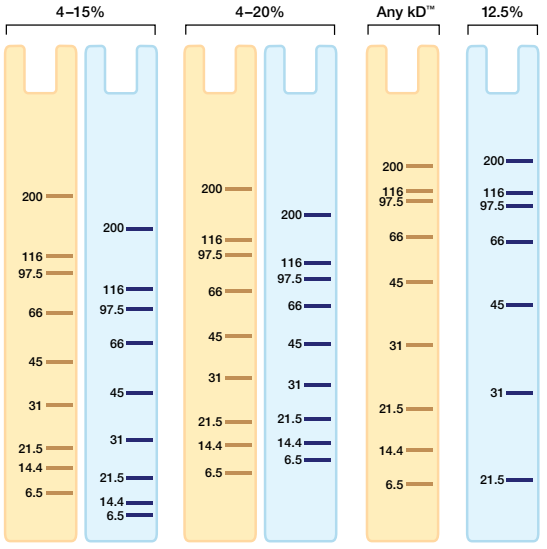
Download the instruction manual at **www.bio-rad.com** and request **bulletin 5507** for ordering information.

Criterion™ TGX™ Stain-Free Precast Gels

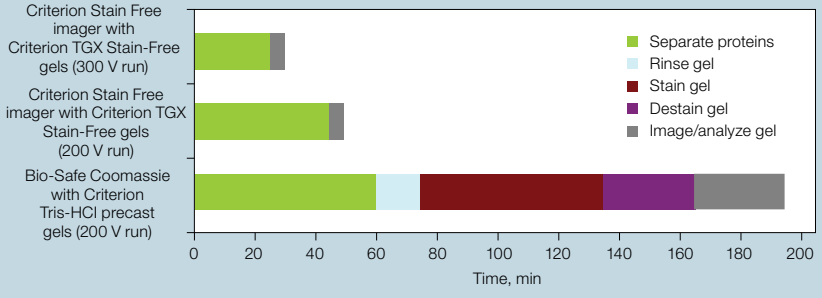
The Criterion TGX (**T**ris-**G**lycine e**X**tended) Stain-Free precast gels for PAGE are based on the long–shelf life TGX formulation and include unique trihalo compounds that allow rapid fluorescence detection of proteins. TGX Stain-Free gels retain Laemmli-like separation characteristics using the standard sample and Tris-glycine running buffers. The proteins in the gel can be separated in as little as 20 minutes and then visualized using the Gel Doc EZ™ imager (see page 16), which offers one-button operation and delivers results in as little as 2.5 minutes.

Benefits:

- Run times as short as 20 min
- Gel images and complete analysis in less than 5 min after electrophoresis
- Inexpensive Laemmli buffer system, low running costs
- Comparable sensitivity to Coomassie stain
- Better reproducibility and quantitation compared to staining procedures
- Accurate molecular weight estimation
- Compatibility with western blotting, standard staining methods, and mass spectrometry



Criterion TGX Stain-Free (■) versus Criterion Tris-HCl (■) gel migration charts. Broad range, unstained standards.



Comparison of Criterion Stain Free™ system and Bio-Safe™ Coomassie staining workflows. Criterion TGX Stain-Free precast gels have shorter run times than the traditional Tris-HCl gels. After electrophoresis, TGX Stain-Free gels take 2.5–5 min to generate results, while Coomassie staining takes at least 2 hr to generate the same level of sensitivity (the graph does not include times for changing solutions).

Ordering Information

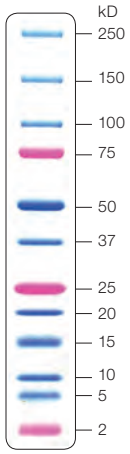
Description	12+2-Well 45 µl	18-Well 30 µl	26-Well 15 µl	Prep+2-Well 800 µl	IPG+1-Well 11 cm IPG Strip
Criterion TGX Stain-Free Gels					
4–15% Resolving Gel	567-8083	567-8084	567-8085	567-8082	567-8081
4–20% Resolving Gel	567-8093	567-8094	567-8095	567-8092	567-8091
Any kD Resolving Gel	567-8123	567-8124	567-8125	567-8122	567-8121

Precision Plus Protein™ Dual Xtra Standards

Precision Plus Protein Dual Xtra standards offer the advantages and features of the Precision Plus Protein family of standards, but also include 2 and 5 kD peptide bands for increased versatility when working with low molecular weight proteins.

Features of the Dual Xtra protein standards include:

- Ability to use one protein standard for both low and broad molecular weight ranges
- 12 prestained bands ranging from 2 to 250 kD
- Sharp, bright, prestained bands for monitoring electrophoresis, western transfer, and accurate molecular weight estimation
- Contrasting pink bands for reference and orientation
- Natural fluorescence properties for multiplexing capabilities
- Lot-to-lot reproducibility in molecular weight consistency and migration



Precision Plus Protein Dual Xtra standard run on a 4–20% Criterion™ Tris-HCl gel.

For more information, visit **www.bio-rad.com/pppstandards**.

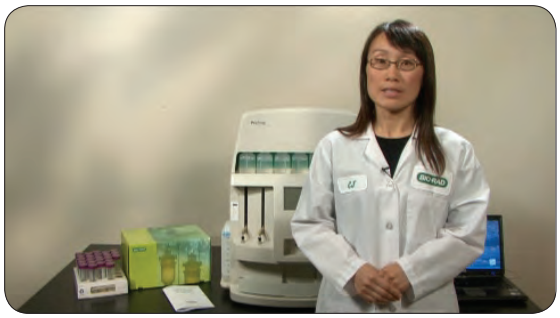
Profinia™ Protein Purification System Demonstration Video Released in Both English and Mandarin

The Profinia protein purification system is an automated affinity purification system that can be used independently for all types of affinity purifications or combined with other comprehensive chromatography instruments as the first step (affinity purification) of tandem purification to achieve high-purity proteins. Affinity purification kits make affinity purification easy, especially with the Profinia system. They can also work well on other purification instruments, or even for any manual purification.

Key features of the Profinia system include:

- Automated operation from column equilibration to cleaning after purification
- Integrated desalting immediately following purification
- Fast purification and easy-to-use features — no need for chromatography expertise

Bio-Rad has released the Profinia protein purification demo video to demonstrate step-by-step operation of the Profinia protein purification system. This video can be viewed on the Bio-Rad website by searching "Profinia demo video".



Profinia demo videos (English and Mandarin) are available on the Bio-Rad website.

To access the Profinia protein purification demo video, visit **www.bio-rad.com**.

For more on using the Profinia system for a variety of affinity purifications, request **bulletin 5541**; for high-purity proteins request **bulletin 5926**; for affinity purification buffers, resin, and cartridges, request **bulletin 5925**.

Bio-Rad Introduces REDFIN 2-D Gel Analysis Service

Bio-Rad is expanding our suite of products to support your 2-D gel electrophoresis experiments. With the REDFIN 2-D gel analysis service available at **www.expressionproteomics.com/redfin**, you can leave spot counting and analysis to us and spend more time on your research.

The REDFIN analysis service is a unique pay-as-you-go system that enables you to buy analysis credits online according to your needs. Results from the service are easy to obtain and ensure both consistency and faster completion times for 2-D analysis.

Features of the REDFIN service include:

- 2-D gel analysis provided by a team of experts
- Secure access to your data on any computer, anywhere in the world
- Online collaboration, data backup, powerful statistics, and many more features at no extra cost
- Can be used as third-party validation of your 2-D analysis results
- Compatibility with all types of staining, including differential in-gel electrophoresis (DIGE)

To view a demo and receive three free credits toward your first analysis project, visit **www.expressionproteomics.com/redfin**.

Use value code: **TRYREDFIN**

Using REDFIN analysis for your 2-D gels is easy:

Step 1: Download the free software at **www.expressionproteomics.com/redfin**.

Simply click the "Download REDFIN" button to start using the REDFIN 2-D gel analysis service.

Step 2: Submit your gel images.

Define your experiment in terms of groups and comparisons, DIGE relations, and technical replicates using the REDFIN software, then upload your 2-D gel images to our analysis center.

Step 3: Select "Basic" or "Pro" analysis service, purchase your credits, and let us do the analysis.

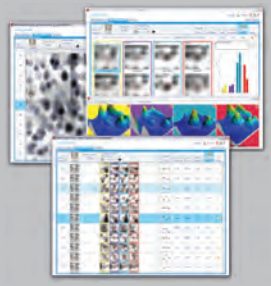
Image analysis is performed by our analysis staff with powerful in-house software and rigid quality controls. The result is top-quality image analysis in a short turnaround time.

Analysis Workflow

1. **Spot detection and segmentation.**
2. **Spot matching.**
3. **Quantitation and normalization.**

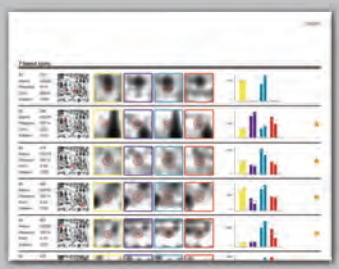
Step 4: Receive your results in a dynamic format.

Filter Results



The results are available in REDFIN software and can be filtered on fold change, *P* value (ANOVA), presence, location, volume, and expression profile.

Export Data



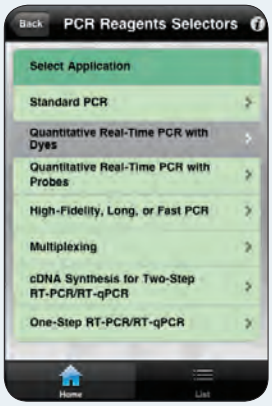
You can easily export your findings to a spot picking file, a PDF report, or an Excel spreadsheet directly from the software interface.

Real-Time PCR iPhone App Updated to Include Reagent Selector Tools

The Bio-Rad real-time PCR iPhone application is an easy-to-use qPCR resource that now includes four helpful tools for researchers performing real-time PCR experiments: the new PCR reagents selectors, qPCR guide, PCR Doctor™, and assay design. The latest version of this application (version 2.0) is compatible with iPhone, iPod Touch, and the iPad, and can be downloaded for free at the iTunes App Store. Once downloaded, the App does not require Internet connection to access or run. Additional features will be added, so please check back for new versions.

New! PCR Reagents Selectors

Choose the best PCR reagent for your specific needs via application or instrument.

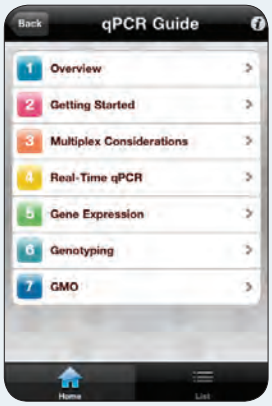


Step 1: Select the application.

Step 2: Select the PCR reagent of interest.

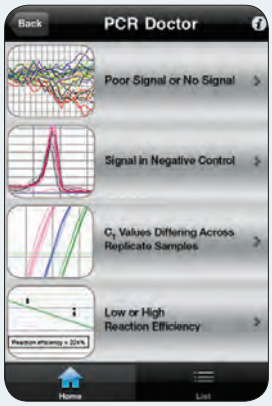
Step 3: Read about the selected reagent and link to the Bio-Rad website.

Other Tools Available



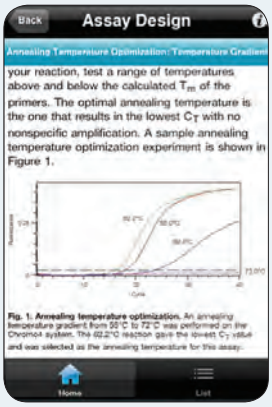
qPCR Guide

Learn more about designing, analyzing, and optimizing real-time PCR experiments.



PCR Doctor

Get help resolving problems related to real-time PCR assays using this interactive troubleshooting tool as well as tutorials on PCR and real-time PCR techniques.



Assay Design

Use these guidelines for designing, validating, and optimizing your qPCR assays, as well as obtaining information about different methods for analyzing qPCR data.

Join Bio-Rad in a "Collaboratory" Effort at Worldwide HUPO 2010

Bio-Rad is a premium sponsor of the 2010 Human Proteome Organization (HUPO), held this year in Sydney, Australia from September 19 to 23. Visit the Bio-Rad booth, meet the proteomics experts, view the posters, and register for the luncheon workshop scheduled on September 20 from 12:15 to 1:30pm.

Poster presentation topics include:

- Fixing Proteomics Initiative — healing the Achilles' heel in 2-dimensional gel-based proteomics
- Comparative protein profile of tissue proteins using a ProteoMiner™ hexapeptide library
- Analysis of large-format 2-D gels in an Alzheimer's disease biomarker discovery study with a mouse model using REDFIN 2-D image analysis service
- Targeted analysis of 2-D gels in an Alzheimer's disease biomarker discovery study with human samples using REDFIN 2-D image analysis service
- Quantitative analysis of plasma samples using REDFIN 2-D analysis service after enrichment using ProteoMiner technology
- Improved sequence coverage and SILAC quantitation of low-abundance proteins from HeLa cell lysates using a ProteoMiner bead library and MudPIT
- Analysis of Alzheimer's disease samples using antibody and chromatographic arrays combined with MALDI-TOF mass spectrometry using the Lucid Proteomics System™
- Phosphoprotein profiling: combining hydroxyapatite-based phosphoprotein enrichment with retentate chromatography MALDI-TOF mass spectrometry using the Lucid Proteomics System
- Clinical sample proteomic profiling: time-course reproducibility of retentate chromatography MALDI-TOF mass spectrometry using the Lucid Proteomics System

Luncheon topics include:

- Improved sequence coverage and SILAC quantitation of low-abundance proteins from HeLa cell lysates using a ProteoMIner bead library and MudPIT
  – Brian Fonslow (John Yate's Lab), Scripps Institute, La Jolla, CA
- High-throughput protein profiling and high-confidence identification for biomarker discovery using the Lucid Proteomics System
  – Stefan Lehr, Institute of Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, Düsseldorf, Germany

Bio-Rad at AOHUPO 2010, Hyderabad, India. Professors Pier Georgio Righetti (far left) from Politecnico di Milano, Verona, Italy and Maxey Chung (far right) from National University of Singapore, gave presentations at the main conference and at the corporate workshop session. Additionally, Professor Righetti autographed copies of his book on proteomics, which was presented to raffle winners (inset).

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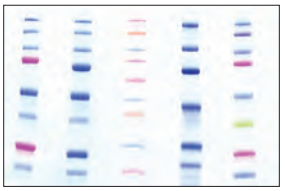
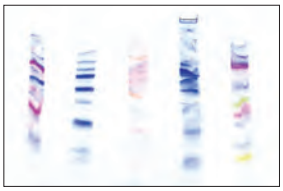
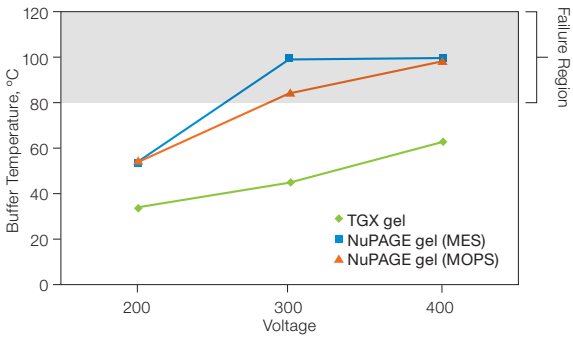


Mini-PROTEAN TGX gel (10%) at 400 V
Results in 12 min



NuPAGE Bis-Tris gel at 400 V
Failure

Mini-PROTEAN TGX (10%) vs. NuPAGE Gel (10%)



Analysis of Clinical Study Variables and Effect on Reproducibility Using the Lucid Proteomics System™

Enrique A Dalmasso and Amanda Bulman
Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction

Protein biomarker development programs center on the discovery, identification, and validation of any differentially expressed proteins and peptides that may serve as biological indicators of altered states resulting from disease, injury, or treatment. Examples include proteins released during cardiovascular injury, shed by or resulting from tumors, or modulated due to drug response. Scientific and clinical interest in novel protein biomarkers and improved panels for existing biomarkers continues to grow, with a goal of better decision making in disease diagnosis and prognosis and in drug discovery applications. Proteomic studies are susceptible to bias from both preanalytical and analytical sources, such that observed differences in protein expression may be a result of bias rather than true biological differences related to the disease or treatment of interest. Care must be taken during study design and the development of study SOPs (for example, sample handling and array preparation protocols) to minimize these biases for increased reproducibility and confidence in results.

In this technical report, we present a series of studies that address various potential sources of variability within clinical biomarker discovery studies and demonstrate their effects on reproducibility of the proteomic profiles obtained using the Lucid Proteomics System.

Methods

Samples

Serum samples were enriched for low-abundance proteins using the ProteoMiner™ protein enrichment kit (Bio-Rad Laboratories, Inc.) as described in the instruction manual, but using a modified elution buffer (7 M urea, 2 M thiourea, 4% CHAPS, 25 mM Tris-HCl, pH >12).

Array Preparation

ProteinChip® cation exchange (CM10) and anion exchange (Q10) arrays (Bio-Rad) were prepared and samples were applied using 96-well ProteinChip bioprocessors (Bio-Rad). The arrays were pre-equilibrated with their corresponding binding and washing buffers: 100 mM NaOAc pH 4.0 for CM10 arrays and 50 mM Tris-HCl pH 9.0 for Q10 arrays. Samples diluted 1:10 (ProteoMiner fractions) or 1:100 (plasma or *E. coli*) in buffer were added to individual bioprocessor wells and incubated for 30 min with shaking at room temperature. Arrays were washed 3x with buffer followed by 2x with deionized water. After drying, 1 µl of 50% saturated sinapinic acid (Bio-Rad) was applied two times to each spot and allowed to dry for at least 60 min before data collection.

Data Collection

Mass spectral data were collected with ultrafleXtreme or ultraflex II MALDI-TOF/TOF instruments (Bruker Daltonics) in linear TOF mode using flexControl software and AutoExecute runs which were defined and exported from Lucid™ proteomics software (Bio-Rad). The Lucid system qualification kit (Bio-Rad) was used to set up, optimize, and test mass spectrometer parameters for reproducible protein profiling in linear mode.

Data Analysis

Raw data were imported into Lucid proteomics software for analysis. Spectra were first grouped into appropriate folders, then processed by baseline subtraction, filtering of electronic noise, setting values for noise calculation, and total ion current normalization. Automatic peak detection was performed using the cluster wizard feature of the software. Within specified mass ranges, individual peaks were labeled across all spectra within a folder and clustered based on their m/z values. Peaks meeting specified user-defined thresholds (in these studies, a minimum signal-to-noise ratio (S/N) of 5 and valley depth of 3) were automatically labeled. Peak clusters were created when a given peak exceeding the threshold was detected in 100% of spectra. Peak intensity coefficients of variation (CVs) automatically calculated by the software were exported and used to calculate the reported median CV values.

Results and Discussion

A series of studies (experimental details summarized in Table 1) addressed potential sources of preanalytical and analytical variability and their effects on reproducibility within biomarker studies. An example of the effect of subtle differences in samples or sample handling (preanalytical experiment) highlights the importance of good study design and the need to control possible sources of preanalytical bias. An example of monitoring the quality control (QC) samples in a real study (day-to-day experiment) emphasizes the advantage of including QC samples within a study to evaluate variability arising from the combination of analytical steps in the Lucid workflow, including sample prefractionation, array preparation, and data generation. The final example (lot-to-lot experiment) emphasizes that the well-controlled manufacturing of ProteinChip arrays minimizes the effects of lot differences as a source of variability across different studies and between results obtained in different laboratories.

Preanalytical Variability

The sensitivity and data-rich nature of proteomic profiling that provides a clear advantage for revealing differences between samples or sample sets for biomarker discovery can also reveal undesired differences — effects of sample handling or differences in the control or stock sample source. Observed differences in protein expression may be a result of preanalytical or analytical bias rather than true biological differences related to the disease or treatment of interest, so all possible sources of

Table 1. Summary of experiments performed to assess variability in biomarker discovery experiments.

Experiment	Sample	Fractionation	Array Chemistry	Bruker Instrument	Figure
Preanalytical	*E. coli* (fresh and frozen)	—	CM10	ultrafleXtreme	1
Day-to-day	QC pool human serum	ProteoMiner technology	Q10	ultraflex II	2
Lot-to-lot	Plasma	—	CM10 (four different lots)	ultrafleXtreme	3

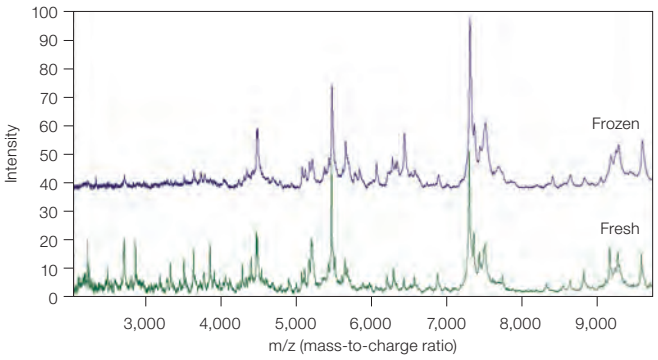


Fig. 1. Proteomic profiles of *E. coli* lysates on ProteinChip CM10 arrays reveal differences between a fresh sample (bottom) and the same sample following one freeze/thaw cycle (top).

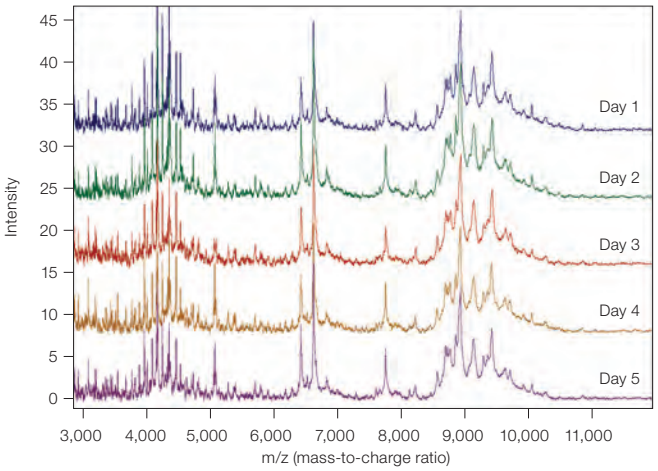


Fig. 2. Reproducibility of proteomic profiles across five different days by profiling ProteoMiner bead–enriched serum on Q10 arrays. Data provided by Dr S Lehr, Institute of Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, Düsseldorf, Germany.

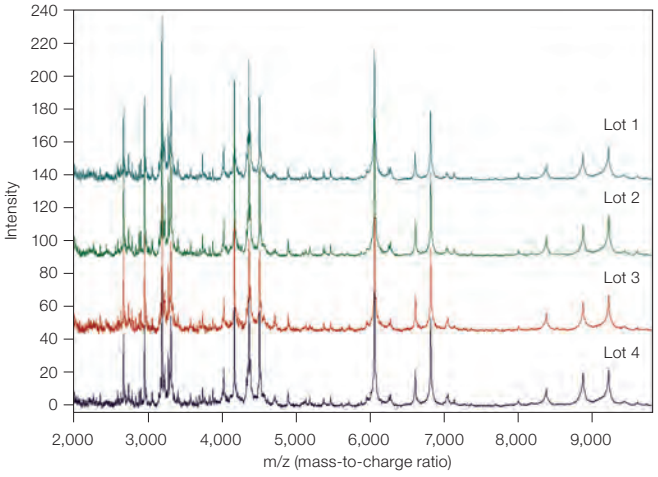


Fig. 3. Reproducibility of proteomic profiles across different lots of ProteinChip CM10 arrays using the same human plasma sample.

bias must be controlled. If not possible to control, univariate and multivariate analyses can help define their possible effects on group classification and determination of biomarker candidates. The effects of preanalytical variability are visible as a loss of a number of low m/z peaks in the representative profiling spectra of an *E. coli* lysate prepared fresh compared to one subjected to a single freeze/thaw cycle (Figure 1).

Day-to-Day Reproducibility

With all analytical measurements, each step in the process contributes to overall performance as measured by reproducibility and accuracy. Therefore, each step must be independently optimized and controlled to generate reliable results. This day-to-day reproducibility example (Figure 2) illustrates that, when combining all analytical steps in the data generation workflow, median CVs of less than 20% can be attained. In this example, sample prefractionation, the various array preparation steps, and linear TOF-MS data generation were all performed following well-defined SOPs, resulting in day-to-day CVs of 10–15% for the three different surface chemistries. This controlled variability for such highly sensitive and data-rich protein profiling provides confidence in biomarker candidates discovered during complex biomarker discovery studies.

Lot-to-Lot Reproducibility

Manufacturing of the ProteinChip array surfaces follows cGMP (ISO90001-2008) practices, including production and QC of starting materials, automation during all manufacturing phases, and in-process and final functional QC. As demonstrated in this lot-to-lot example (Figure 3), the reproducibility attainable across lots of arrays manufactured over a two-year time period is comparable to the day-to-day reproducibility when repeating the same experiment using single lots of arrays, and was 16% for this example. This enables and supports the performance of experiments over a period of time or between different laboratories utilizing the Lucid Proteomics System in biomarker research applications.

Conclusion

We have shown that uncontrolled preanalytical bias can contribute significant variability to proteomic profiles and that median peak intensity CVs of less than 20% can be attained for QC samples processed alongside study samples. The parallel processing of a significant number of QC samples within a biomarker study is imperative in order to monitor variability caused by the combination of individual analytical steps in the proteomic profiling workflow. Also, the consistent and reproducible proteomic profiling performance of different lots of arrays significantly increases the confidence in clinical studies performed at different periods of time and when comparing results across clinical studies performed by different laboratories.

For more information, request bulletin 6010.

SIMPLICITY IN IMAGING: FROM CONCEPTION TO LAUNCH

By Shawn Miller

Renee LeMaire-Adkins, PhD
Division Marketing Manager

Product Conception

Bio-Rad employees — regardless of department or role — are encouraged to recognize needs not being met in the life science research market and weigh in on ideas for new product development. In the case of the Gel Doc EZ imager, it was the inspiration of a team of Bio-Rad specialists to provide high functionality without complexity for gel electrophoresis imaging applications that led to the system's development. Renee LeMaire-Adkins was among those on this original team.

Following a research career using a variety of imaging technologies — from the early days of microscopy and film to digital imaging to confocal and deconvolution systems — Renee started at Bio-Rad in Technical Support, specializing in imaging products. This position quickly led to one as a product manger for camera-based imaging devices, which eventually led to her being named division marketing manager for the entire imaging products line. To the right, Renee describes the rationale behind the development of the Gel Doc EZ imager.

Many concepts — like the Gel Doc EZ imager — being worked on right now are a natural evolution of thinking that was going on 10 years ago when I started with the imaging group. A lot of researchers find it either intimidating to learn to use complex imaging and blotting systems, or frustrating to have to remember how to use them. The drive toward one-button operation was motivated by the realization that people purchase systems and don't always continue to use them because they can't remember how. If researchers can't remember how to collect a good image or picture of their gel or blot, then they've lost their experiment — particularly when it comes to chemiluminescence. We know that in many labs, there is usually one "expert" who knows the ins and outs of how to use an imaging system, but once he or she moves on, others become frustrated because they are not able to operate the equipment. Over time, we began to realize that even basic gel documentation systems tend to be more complicated to use than they should be and then become underutilized.

One of the reasons that many gel imaging systems out there are painful to use is because they are based on operating a manual camera — meaning that the researcher must know how to set aperture, film speed, and exposure time.

Introduction

When it comes to gel imaging and blotting, conventional imaging devices have tended to offer either high-performance, complex functionality or low-cost, low-performance simplicity. That is, until now. Bio-Rad has developed a one-button gel imaging system — the Gel Doc™ EZ system — that allows customers to image everything from gels to more complex applications automatically. This general-purpose, versatile instrument has a compact footprint, making it a space-saving, economically viable choice that does not compromise on performance. These streamlined features — both in size and operation — make the Gel Doc EZ imager ideal for the current laboratory environment in which efficient use of resources is critical.

The development of this system represents the culmination of years of progress toward simplifying the imaging step of gel electrophoresis workflows. As with all new product releases, launching the Gel Doc EZ system has required the vision and expertise of a cohesive internal team as well as testing and feedback from the scientific community. The key players involved in bringing the Gel Doc EZ system to market describe, in their own words, the critical steps — from conception to validation to product launch — involved in development of this latest imaging innovation.

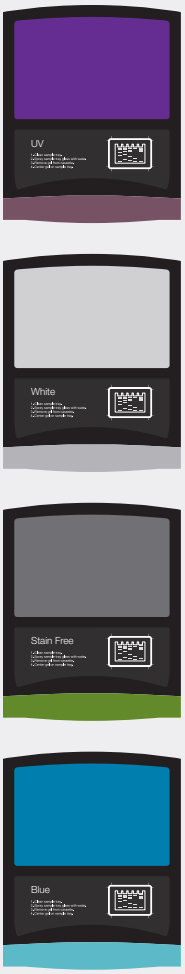
Think about the general population and the number of people who have never touched a manual camera and know how to operate only the automated cameras we have today. Much like mainstream camera counterparts, one-button image-capture operation for the life science community became a major priority in terms of product development.

As the imaging group was moving in the direction of one-button operation, a dialogue began with the electrophoresis group regarding difficulties associated with staining and destaining protein gels. We thought, “Wouldn’t it be great to not have to do this?” Working together with the electrophoresis team, we developed Criterion™ Stain Free gels and the Criterion Stain Free™ imaging system. The launch of the Criterion Stain Free system was quickly followed by the development and launch of the TGX™ long shelf life gels. The introduction of this line started additional dialogue around the possibilities of broadening the capabilities of the stain-free imaging device. So what we have done with the Gel Doc EZ system is take the simplicity that was pioneered with the Criterion Stain Free platform and apply it more broadly to general gel and blot imaging applications.

Suresh Mehta
Senior Mechanical Engineer

Hardware Development

Upon validation of an instrument product idea, it becomes the job of the hardware engineer to render the concepts tangible. Suresh Mehta's mechanical engineering background gives him strength in precision mechanical design and optomechanics. During his tenure at Bio-Rad, Suresh has worked on Gel Doc imaging system enhancement projects, the ProteOn™ protein interaction array system, and his latest hardware engineering project, the Gel Doc EZ system. Suresh describes the process and challenges faced in the engineering step of Gel Doc EZ imager development.



Gel Doc EZ imager's application-specific trays. Choose from UV, blue light, stain-free, and white light application trays. The instrument senses which tray has been inserted and images the sample according to settings optimized for each application.

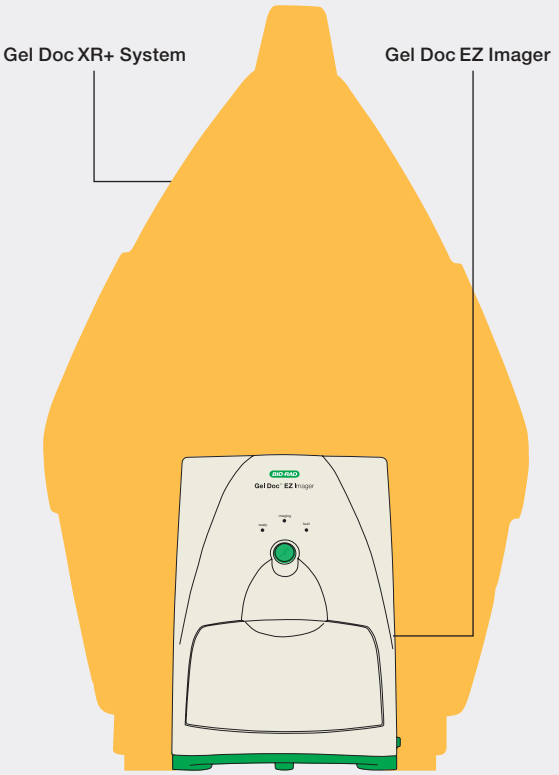
The way our team is structured, we all interact and collaborate from the conceptual stages of a new product to delivering the instrument to manufacturing. When developing specs for the product, we work closely with internal customer-facing personnel (marketing and systems integration groups) as well as solicit feedback externally from potential customers regarding their needs to determine what the product must be able to do. When the Gel Doc EZ system team was formed, I was assigned as the hardware element of the team.

From a functional perspective, the primary goal of the Gel Doc EZ project was to provide a version of our very successful Gel Doc system in a smaller, more user-friendly package. From a hardware engineering perspective, this project was based on the existing Criterion Stain Free platform, so we were able to start with an existing "envelope." Not starting from the ground up was a big plus. The standard Gel Doc imaging system is designed for a platen that is 25 x 26 cm, so it accommodates very large gels. The Gel Doc EZ platen is much smaller at 15 x 11.25 cm. By reducing the size and the footprint of the instrument — while providing enhanced image capture and analysis capabilities — we've simplified the post-electrophoresis user experience.

This new instrument doesn't require manual camera adjustments; it's a single-button operation, so the customer doesn't need familiarity with the instrument to take great looking images. The Gel Doc EZ system uses Image Lab™ software — a much improved and enhanced user-friendly software compared to what had been available before.

Because of the emphasis on simplicity for the researcher, we created four application-specific trays for the device. This was the most challenging part of the project from a design perspective — it took a little bit of, shall we say, inspiration and innovation to be able to do that. These trays interact with the instrument. The researcher simply places his or her gel on the tray and inserts it into the instrument. The instrument recognizes which tray it is and then performs the application specific to that tray type. Some examples of these applications include ethidium bromide (most common application for imaging instrumentation), SYBR® Safe (for nucleic acids with the advantage that it doesn't destroy DNA structure by illumination at the time of imaging), stain-free gels, and Coomassie gels (gray-scale imaging).

In the design of the trays, we also accounted for the size of Bio-Rad gel products so we can image the gels in their own trays (on top of our trays) without researchers needing to transfer and potentially tear the gels.



The Gel Doc EZ imager offers the flexibility and functionality of complex imaging systems but in a much more compact, easy-to-use platform.

Keith Kotchou
Senior Software Engineer

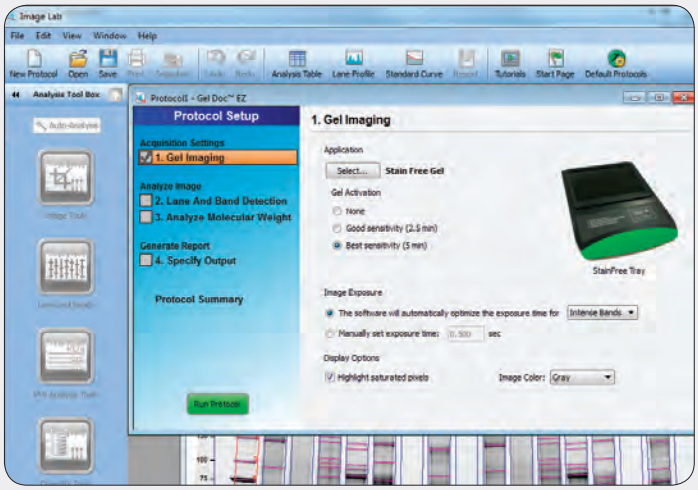
Software Development

No instrument can realize its potential without a software package that is optimally designed to operate system firmware. Keith Kotchou started his software development career with a company that creates CCD cameras, so even before he joined Bio-Rad's imaging group 17 years ago he had acquired extensive knowledge about cameras and imaging. Keith remembers a time when electrophoresis gel imaging instruments were not camera based but instead were scanners that required the use of radioactive materials. Over time, the systems improved and evolved in capability, and throughout that time Keith worked to develop higher-level user software to operate each instrument introduced by Bio-Rad.

My involvement with the Gel Doc EZ imager project began with the Criterion Stain Free system in 2007. The idea for the Gel Doc EZ imager has been in the background for a while but moved to the forefront with the success of Criterion Stain Free system. In all imaging system projects, I am involved a little bit with conceptual stages, then as firmware gets developed, I get together with the firmware designer to discuss software requirements and figure out various commands.

The biggest challenge with this particular project was presenting high-level software features to the user in a friendly, intuitive way. It can be tempting to include every bell and whistle in the user interface of a software program, but then you add complexity to the process, so the real challenge can be paring down and narrowing the focus of the program so that it's simple to use but still includes both basic and complex functionality. Because the hardest part — from a software development point of view — is coming up with a simple-to-use interface, it's important to recruit alpha and beta users (customers in the field). We watch what they do, take notes, have them do surveys, then adapt and change the software based on actual researcher usage.

A unique feature of this software compared to programs I've developed for other systems is that it is also translated into Mandarin. To do this, we had to go through the application and isolate the text strings that need to be translated from those that are internal to the program (things we can't change or the program won't work). This required wading through hundreds of strings — it can be a mind-numbing process. The translated strings are embedded in the software, so the regional settings on the customer's computer will determine if the software displays in English or Mandarin. For customers, this process is seamless. They don't have to do anything but run the software on their system to have it display in their desired language.



Software developed for the Gel Doc EZ imager displays in either English or Mandarin depending on the user's regional computer settings.

Kevin McDonald, PhD
Senior Staff Scientist

System Validation

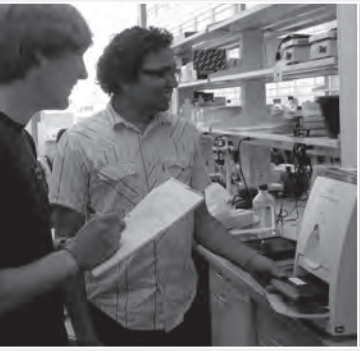
Once hardware and software requirements have been defined and developed, early-version imaging systems must then pass the rigors of internal validation procedures. Kevin McDonald is the main systems validation person for all imaging-related hardware and software projects. A cell biologist by training, he started his career at Bio-Rad in Technical Support. Before the Gel Doc EZ project, he worked on the Gel Doc, ChemiDoc™, Pharos FX™, and GS-800™ systems, among many others. As he says, "I was a customer for many years as a scientist, then in Tech Support I got to come across problems vicariously through others. My job is to make sure that the software and hardware are doing what the application needs to do from the perspective of a scientist who is using the instrument. So I'm basically the voice of the customer in-house."

Our imaging products aren't pieces of hardware and software; they're instruments designed to give information of value to scientists. So in testing these instruments, you need someone who understands what gels are, what scientists are trying to do with the gels, what information they're looking for, and how they use gels in their normal fields of study.

With systems validation, you have a project plan and you test to that plan. Sometimes you can't meet the specifications in that plan so you have to figure out why. Maybe the specifications were not defined appropriately to begin with; sometimes you have to go back and retest in case there was a bad sample. Other times there are software issues that pop up. When conducting validation testing we try to be as close to the customer experience as possible and always evaluate to make sure the customer obtains the best results possible. Sometimes we'll have a situation where something technically matches the test plan requirements but I'll see something that I think could improve the product, so we will go ahead and fix it even though the product technically passes validation check. You always keep your eye open for things that may affect the customer's perception of the product or their ability to use it.

Some of the challenges faced in the Gel Doc EZ project had to do with settling on an exact hardware setup. I'll say to the engineers, "OK, we need to look at this particular application, which is going to require this excitation source and this emission filter." So they implement that to the best of our estimates as to what is required. Then we have to run the applications and evaluate a number of things: appearance of the image, accuracy of the data, that sort of thing. Depending on a balance of how things work out for all the applications we evaluate, we have to change things. So we do. We've changed some filters on the excitation sources; we've changed emission filters to improve performance, both in image quality and in quantitative accuracy of the data.

The voice of the customer is something we pay very close attention to in the system validation process. In alpha and beta stages of development testing, we have a set of customers who are giving us feedback. We establish direct email lines of communication from these customers to the core product teams for hardware, software, systems integration, and marketing. The customers can email directly to the team any issues or questions, and we can respond very rapidly. Then we go back and rework software or hardware — whatever is needed to address problems. The ultimate challenge is the unlimited permutations that people have in terms of samples and how they are used in combination with our hardware and software. It's impossible to test for the infinite possibilities customers might have. So then we just try to be as responsive as possible to any issues that might arise.



Customer Feedback Dr Sriram Kosuri is a post-doctoral candidate in the George Church laboratory at Harvard University. Widely renowned for its ground-breaking work in gene synthesis, genetic sequencing, and in the Human Genome Project, the lab relies heavily on imaging technology to visualize and analyze DNA and RNA gels (primary applications) as well as screen colonies. Kosuri estimates that researchers in this lab run 5–10 gels per day, 98% of which are basic nucleic acid gels.

With several imagers at its disposal, including one by AlphaInnotech and a more sophisticated chemiluminescent imager, the lab recently acquired the Gel Doc EZ imager. Kosuri says, "The Gel Doc EZ imager has really been gaining traction in terms of usage in our lab. It's a very nice machine that's easy to use for basic agarose/PAGE gels." So easy, in fact, that of the dozen or so researchers who have started using the imager — many of whom are new to the Church lab — none required training. "People appreciate not having to fuss with focus and aperture," he says. And though it's simple to operate, there is no need for the Church lab to sacrifice quality. "We just submitted an article for publication that includes many images obtained on the Gel Doc EZ imager," says Kosuri.

Nik Chmiel, PhD
Staff Scientist, Systems Integration

A Prelaunch User's Perspective

Before placing a newly developed imaging system in the hands of life science researchers, Bio-Rad continues to optimize performance based on feedback from internal customers. After finishing a post-doc at the University of California, Berkeley studying RNA-protein interactions, Nik Chmiel joined Bio-Rad as a systems integration scientist in gel electrophoresis and blotting. The nature of his work at Bio-Rad made him an ideal internal customer to test and provide feedback on the functionality of the Gel Doc EZ system prior to its release in the marketplace. Nik describes his experience using the imager in various gel electrophoresis experiments over a two-week period.

I was approached by the imaging team, who told me they developed a new instrument (the Gel Doc EZ system) and asked if I would mind acting as an internal beta tester for the system. I agreed and was enthusiastic about the opportunity to "kick the tires" on the team's newest concept. Installation was very straightforward: the imaging team brought the system over, plugged it in, connected it to the laptop, and within a few minutes I was up and running.

In the past, I would use the GS-800 calibrated densitometer in a typical gel imaging workflow or the Gel Doc system for white-light imaging applications like Coomassie-stained protein gels or for UV applications, such as visualizing ethidium bromide–stained DNA bands on agarose gels. I've also used other multi-use systems that contain either separate UV and white-light sources or can convert UV to white light using a special screen placed on top of the UV transilluminator. The common thread of all of these systems is that they require you to adjust several settings, like lens zoom, focus, and aperture, every time you change the size of your gel or the type of image you want to take. This process can be time consuming, especially the first couple of times you use the instrument. In addition, it can be frustrating to have to remember the particular settings you used for an image, especially if there are multiple users of the system, all with their own particular imaging setting requirements.

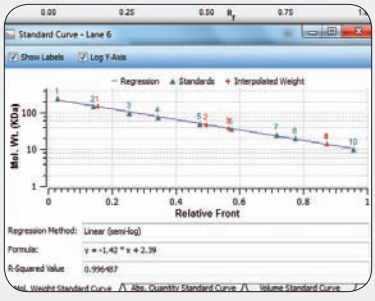
My overall experiences with the Gel Doc EZ system were very positive. I was able to figure the system out in just a few minutes of tinkering with the software and appreciated the fact that I could select an application and press a single button while the instrument did everything else — including data analysis with all useful parameters of results that I could export into practically any file format. There was no fussing with the lens focus or integration time; all the parameters I would have to adjust on other imagers were automatically optimized. Performance was also similar to other systems I've used, but the footprint was much smaller. This is great in a lab environment, where every square foot of space on a lab bench is precious. Its compact size also allows the Gel Doc EZ imager to be placed in an easily accessible location, such as the end of a bench, rather than in a separate room or off to the side somewhere, as is usually required with larger imagers.

The imaging team was very receptive to my feedback on their system. One change I requested was the ability to image Bio-Rad precast agarose gels in their trays. These trays contain features that allow for easy sample identification on the gels. In a previous Gel Doc EZ prototype, the gels worked just fine but the trays were a bit too tall to fit in the imager. The team incorporated my suggestion and the current prototype creates great agarose gel images — trays and all.

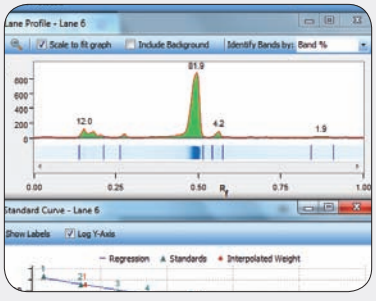
Conclusions

The Gel Doc EZ imager is the culmination of years of work toward eliminating the complexity associated with the imaging step of the electrophoresis workflow. Much as the advent of point-and-click photography in the consumer market allowed practically anyone to create professional-quality photographs, the development of one-button imaging for a variety of electrophoresis applications is poised to eliminate operator inexperience and error as factors preventing capture of high quality, reproducible gel images.

A



B



C

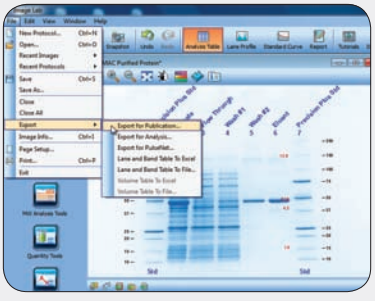


Image Lab software automatically detects lanes and bands, then performs analyses such as standard curves (A) and lane profiles (B), and compiles data tables of results. Reports generated can by exported to practically any file format (**C**).

Normal Physiological Levels of Human Cytokines Using Bio-Plex Pro™ Cytokine Assays

Philip Chapman, Candice Reyes, and Vinita Gupta
Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction

Cytokines function as indicators of inflammation or disease progression and provide a means of manipulating cellular responses in vivo and in vitro. In healthy individuals, cytokines are expected to have low or undetectable circulating levels, whereas they have been shown to be elevated in a number of disease states. As a result, the ability to detect these factors has become increasingly important to researchers and clinicians. The objective of this study was to determine the range of cytokines in serum collected from apparently normal donors that could be used as reference in clinical studies and to describe the preparation of samples for optimal use with the Bio-Plex® suspension array system. In previous published studies, difficulty in obtaining accurate values has been reported due to variations in sample collection, processing, storage, and day-to-day operation. Therefore, these ranges should be used only as a guideline.

Methods

Human serum samples from 66 normal donors of all ages and ethnicities were obtained from Bioreclamation, Inc. and from the Bio-Rad Laboratories, Inc., Redmond, Washington facility. Two different vendors were chosen to avoid bias in calculating concentration ranges contributed by variations in blood collection, processing, and storage conditions. Samples were tested with human group I and group II Bio-Plex Pro cytokine assays. The samples were measured in duplicate at a low PMT setting using directions provided in the instruction manual. The assays were performed using the Bio-Plex Pro II wash station with the magnetic plate carrier to minimize operator-related variations. A 10-point extended broad range standard curve was used in order to maximize sensitivity for samples containing very low levels of analytes. High-end saturation points were removed from the standard curves for determining sample concentrations.

Results

Most of the cytokines had mean values <100 pg/ml with few exceptions. IL-1β, G-CSF, and β-NGF were not detected in most of the samples tested due to low circulating levels under normal physiological conditions (<1.5 pg/ml). PDGF-BB, RANTES, and SCGF-β had high endogenous levels and may require higher dilution of samples from diseased states. In general, group II cytokines have slightly higher endogenous levels compared to group I cytokines.

Table 1 shows the concentration in range, observed concentration range, and median and mean concentrations. The concentration in range column provides only observed concentration values that fall within the range of valid standards that can be estimated accurately (with 70–130% recovery). The observed concentrations correspond to positive extrapolated data or data above or below the fluorescence intensity of standards and these values do not necessarily fall within the assay working range. Calculated sample values below the standard curve range are included to demonstrate sample sensitivity of the assay. However, these values should not be considered reliable as they fall outside the standard curve. The mean and median correspond to the mean and median of concentration in range values for the 66 samples tested and includes any samples with undetectable levels. All values take into account the 4-fold dilution factor used when screening the samples.

Table 1. Normal physiological levels of group I and II human cytokines.

	pg/ml			
Analyte	Concentrations in Range	Observed Concentration	Median of Concentrations in Range (n=66)	Mean of Concentrations in Range (n=66)
Group I Cytokine Assays				
Basic FGF	4.00–55.00	1.30–55.00	7.54	9.00
Eotaxin	2.00–39.00	1.20–39.00	0.00	3.80
G-CSF	<1.50	<1.50	0.00	0.02
GM-CSF	3.00–122.00	0.80–122.00	6.78	12.47
IFN-γ	7.00–124.00	0.60–124.00	8.68	13.43
IL-1β	<0.70	0.02–0.70	0.00	0.01
IL-1ra	6.00–665.00	0.20–665.00	23.94	42.01
IL-2	2.00–90.00	0.03–90.00	1.24	6.46
IL-4	0.06–3.00	0.01–3.00	0.00	0.10
IL-5	1.00–7.00	0.01–7.00	0.00	0.15
IL-6	0.50–9.00	0.02–9.00	0.00	0.73
IL-7	0.60–13.00	0.01–14.00	0.00	0.27
IL-8	0.40–116.00	0.08–116.00	0.00	7.21
IL-9	2.00–500.00	0.38–500.00	19.40	37.50
IL-10	0.40–2.00	0.10–2.00	0.00	0.13
IL-12(p70)	3.00–6.00	0.10–6.00	0.00	0.14
IL-13	0.80–9.00	0.01–9.00	0.00	0.33
IL-15	2.00–5.00	0.06–5.00	0.00	0.31
IL-17	2.00–31.00	0.22–31.00	0.00	2.30
IP-10	6.00–637.00	5.90–637.00	32.24	93.61
MCP-1 (MCAF)	2.00–48.00	2.00–48.00	17.95	18.24
MIP-1α	<2.00	0.01–2.00	0.00	0.15
MIP-1β	5.00–47.00	1.70–47.00	11.24	14.75
PDGF–BB	6.00–3,667.00	6.00–3,667.00	180.10	394.87
RANTES	100.00–2,282.00	100.00–2,282.00	0.00	203.64
TNF-α	6.00–98.00	0.10–98.00	0.00	5.92
VEGF	0.50–9.00	0.01–9.00	0.00	0.43
Group II Cytokine Assays				
CTACK	1.00–1,086.00	1.00–1,086.00	196.00	246.51
GRO-α	9.00–365.00	9.00–365.00	22.35	36.33
HGF	63.00–1,868.00	63.00–1,868.00	195.20	255.24
IFN-α2	14.00–79.00	3.30–63.00	0.00	16.07
IL-1α	0.50–1.40	0.40–1.40	0.00	0.12
IL-2Rα	28.00–594.00	28.00–594.00	102.66	116.85
IL-3	13.00–170.00	13.00–170.00	41.06	44.54
IL-12(p40)	36.00–646.00	36.00–646.00	0.00	60.19
IL-16	10.00–1,270.00	10.00–1,270.00	77.50	94.79
IL-18	9.00–812.00	9.00–812.00	68.05	75.71
LIF	4.00–55.00	4.00–55.00	14.85	17.20
MCP-3	1.00–78.00	1.00–78.00	2.25	3.28
M-CSF	6.00–208.00	6.00–208.00	29.64	48.68
MIF	6.00–2,003.00	6.00–2,003.00	72.40	170.29
MIG	86.00–7,911.00	86.00–7,911.00	289.00	617.05
β-NGF	<1.10	<1.10	0.00	0.04
SCF	16.00–837.00	16.00–837.00	167.57	172.88
SCGF-β	6,054.00–130,932.00	6,054.00–13,0932.00	47,870.00	48,312.25
SDF-1α	8.00–92.00	8.00–92.00	0.00	13.79
TNF-β	1.00–13.00	0.71–13.00	0.00	0.31
TRAIL	8.00–272.00	8.00–272.00	66.61	65.91

Conclusion

No significant differences were observed in sample analyte measurements from different vendors. Cytokines such as IL-8, IL-15, and IL-17 have previously shown variability in concentrations obtained from samples collected by different vendors. As mentioned above, this variability could be due to differences in blood collection method, storage conditions, and freeze-thaw cycles of samples. Therefore, it is advisable to use these values as a reference. It is recommended that the reference samples and the samples under study be collected and stored under identical conditions. Refer to suggested reading for additional information.

Suggested Reading

Blood collection, storage, and processing:
Aziz N et al. (1999). Variables that affect assays for plasma cytokines and soluble activation markers. Clin and Diag Lab Immun 6, 89-95.

Normal cytokine ranges in plasma:
Kokkonen H et al. (2010). Up-regulation of cytokines and chemokines predates the onset of rheumatoid arthritis. Arthritis & Rheumatism 62, 383-391.

Evaluation of Oriole™ Fluorescent Gel Stain for Use in 2-D SDS-PAGE to MALDI-TOF Workflows

John Walker II, Katrina Academia, Tom Berkelman, Xiaoyi Xu, and Aran Paulus
Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction

Fluorescent gel stains are an important class of reagents in protein analysis because of their high sensitivity, wide dynamic range, ease of use, and compatibility with downstream analytical processes such as mass spectrometry (MS). Despite the advantages over traditional stains such as Coomassie brilliant blue (CBB) and silver stain, fluorescent gel stains do have several drawbacks including the need for a high-end imager featuring LED or laser excitation in the visible range as well as lengthy staining and processing times. Recently, Bio-Rad introduced Oriole fluorescent gel stain, a novel reagent for staining protein gels in a one-step procedure that allows optimal imaging using a wide range of UV illuminations (Berkelman and Walker 2009). The strong absorbance of Oriole stain over a broad UV range allows visualization and excision of spots from gels without the need for counterstaining. Circumventing some of the general drawbacks associated with most fluorescent stains expedites sample processing and lessens the waste typically associated with the use of additional reagents. The entire staining procedure, including imaging, can be completed within 2 hr. Furthermore, the gel can be imaged using a relatively inexpensive UV imager. This technical report presents the results of preliminary investigations into the utility of staining 2-D gels with Oriole stain and subsequent mass spectrometric analysis.

Materials and Methods

All materials used are from Bio-Rad Laboratories, Inc., unless otherwise noted.

Isoelectric Focusing and Gel Electrophoresis

2-D SDS-PAGE standards were diluted at 15 µl/ml into an isoelectric focusing buffer. This sample was used to rehydrate six 11 cm ReadyStrip™ IPG strips, pH 3–10 NL (200 µl/strip). Samples were passively rehydrated on a PROTEAN® IEF cell and then focused for a total of 35,000 V hr. The strips were then treated first with ReadyPrep™ equilibration buffer I for 20 min at room temperature, then alkylated for 20 min using ReadyPrep equilibration buffer II (containing iodoacetamide). The strips were assembled onto the tops of precast Criterion™ Tris-HCl 8–16% gels. The gel cassettes were loaded into a Criterion™ Dodeca™ cell and electrophoresis was performed for 55 min at a constant 200 V. At this point, the gel cassettes were disassembled and the gels subjected to the various staining procedures.

Gel Staining

Gel staining was performed at room temperature following manufacturer's guidelines. Colloidal Coomassie staining was performed according to the method of Neuhoff (Neuhoff et al. 1988) using Bio-Rad reagents and washed with water prior to imaging. SYPRO Ruby–stained gels were fixed with 40% ethanol/10% acetic acid for 60 min and stained overnight. Destaining was performed using 10% methanol/7% acetic acid for a total of 60 min. Gels stained with Oriole stain were transferred directly from the gel cassette to the staining solution. Staining was performed for 90 min.

Imaging

Fluorescent gels were imaged on a Molecular Imager® VersaDoc™ MP 4000 system. Imaging was conducted using the software-supplied parameters for each stain. Settings for SYPRO Ruby were also used for the samples stained with Oriole stain. The resulting images were processed and subjected to analysis using PDQuest™ 2-D analysis software. Results reported are the average values from three gels. Automatic spot counting was performed and the results confirmed manually. Standard spot numbers (SSP) were assigned to the matched spots common to all gels analyzed to produce a master gel. Spot volumes were summed and combined for each protein area. The combined volumes are expressed relative to the whole.

Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Analysis

Following imaging, the fluorescently stained gels were counterstained using Bio-Safe™ Coomassie stain. The gels were then prepared for automatic spot excision using an EXQuest™ spot cutter. The counterstained gels were placed on the cutting surface, imaged, and samples excised using a white light source. In parallel, gels stained with Oriole stain were placed directly on the EXQuest spot cutter and samples were excised following UV epi-illumination. Samples were automatically distributed into 96-well protein purification plates (Thermo Fisher Scientific, Inc.) for processing on a Freedom EVO 100 liquid handling robot (Tecan Group Ltd.). The plugs were destained, dehydrated, and each plug was subjected to tryptic digestion (Promega Corporation) overnight. The digest solution was centrifuged into clean plates and the plugs further treated with 0.5% v/v trifluoroacetic acid (TFA) for 30 min. This was filtered and combined with the original filtrate to give ~10 µl of sample solution at pH ~2. The acidified digested samples were prepared for MALDI-TOF analysis by the dried droplet method using α-cyano-4-hydroxycinnamic acid (CHCA, Sigma-Aldrich). Upon drying, the sample plate was introduced into the mass spectrometer. MALDI-MS was performed on a 4800 Plus MALDI TOF/TOF analyzer (AB SCIEX) in MS-MS/MS mode using the 4000 Series Explorer software (v3.5.3, AB SCIEX). A total of 400 shots/sample were acquired in positive, reflector mode over the 750–3,500 Da range for MS processing. The five most intense signal peaks from the digest were then subjected to MS/MS analysis using 2 kV collision energy and combined with the parent MS data for database searching. Peak lists were created

and submitted to the Mascot database search program (Matrix Science Ltd.) using GPS Explorer software (v3.6, AB SCIEX). Only results with a probability of randomness of <0.05 (p <0.05) were considered significant and included in this evaluation. Results are presented in regard to peptides matched and protein sequence coverage achieved.

Results and Discussions

MALDI-MS, in conjunction with TOF detection, is ideally suited to study peptides derived from digested proteins. Protein identification through peptide mass fingerprinting is the most common application in this regard. Additionally, the MALDI process allows automation with high sample throughput and, when equipped with the capability to perform tandem MS, the ability to interrogate the molecule for posttranslational modifications. Limitations to MALDI include a limited dynamic range within a sample (about 2 orders of magnitude) and difficulty in analyzing mixtures within a sample. For these reasons, proteome analysis via MALDI often utilizes the resolving powers of 2-D SDS-PAGE for the initial separation and isolation of proteins and complexes prior to proteolytic digestion.

Silver nitrate is the benchmark for sensitivity in protein gel staining, though its dynamic range is often only 1 log order and can inhibit MS analysis. Numerous techniques and products have been developed to overcome or lessen these detriments (Lin et al. 2008) and better alternatives exist, such as fluorescent stains (White et al. 2004). These stains, including Oriole stain and SYPRO Ruby, have been shown to offer nanogram-level sensitivities, linear dynamic ranges of 3 log orders, and to be compatible with MS analysis (Ball and Karuso 2007, Berkelman and Walker 2009, White et al. 2004). In our studies, it became apparent that the Oriole stain may be ideally suited for MALDI-MS identification of proteins.

One important aspect of this sample analysis workflow is the harvesting of protein spots of interest from the 2-D gel prior to proteolytic processing. CBB counterstaining of fluorescently stained gels is a common way of visualizing proteins prior to their excision from the gel. The sensitivity of CBB can be up to 100-fold less than that of fluorescent stains, though it is thought to be within the range of MALDI detection limits. However, CBB-stained spots, especially the low-abundance proteins, do not always offer a high-contrast image. Spot-cutting alignment, either by eye or by using software, can fall off in these analyses. While counterstaining does add time to an already lengthy workflow, cutting CBB-stained spots requires only a white light source to aid vizualization. White light and UV illumination options are generally part of most automated spot excision systems. Therefore, we explored the use of Oriole staining for image analysis as well as mapping for spot cutting. Oriole stain has a strong absorbance in the UV range providing a high-contrast image that makes counterstaining unnecessary.

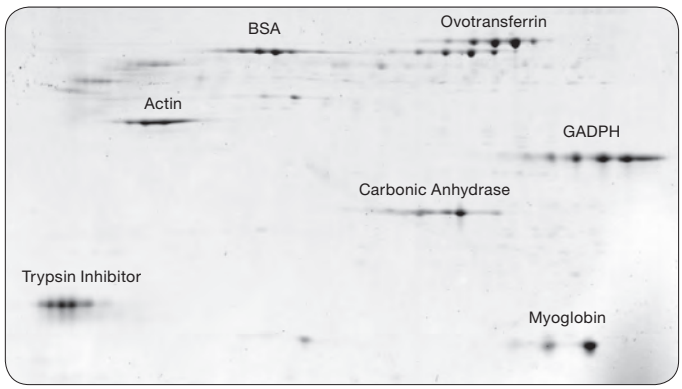
Staining and Image Analysis

In this study, we used a simple 2-D SDS-PAGE standard containing seven proteins and their isoforms across a range of isoelectric points (pIs) and molecular weights (MWs). Staining using either SYPRO Ruby or Oriole stain produced high-quality images with little background interference using a VersaDoc 4000 MP system. Spot counting was performed automatically using PDQuest software and confirmed by manual inspection of the assigned locations. SYPRO Ruby and Oriole staining allowed 42 and 41 spots to resolve, respectively, while CBB staining identified an average of 37 spots (data not shown).

Using the known pI/MW values for each protein, the stained isoform regions can be assigned to one of each of the proteins (Figure 1A). Combining the spot volumes from the individual locations into protein groups allowed us to look at staining preferences (Table 1). Oriole stain and SYPRO Ruby produced consistent results. There was a strong preference for the binding of the stains to ovotransferrin and GADPH, which accounted for about 50% of the total spot volume. SYPRO Ruby does stain the trypsin inhibitor more readily than Oriole stain, while the opposite is true for BSA. In this experiment, each stain exhibited an average coefficient of variance between spots of around 13% across the seven proteins.

These results, and those from earlier studies (Berkelman and Walker 2009), show that Oriole stain has the same desirable properties as other fluorescent protein stains: nanogram level sensitivity, linearity of response over a wide range, and compatibility with UV-based gel imaging. The advantage of Oriole

A. VersaDoc Imager



B. EXQuest Spot Cutter

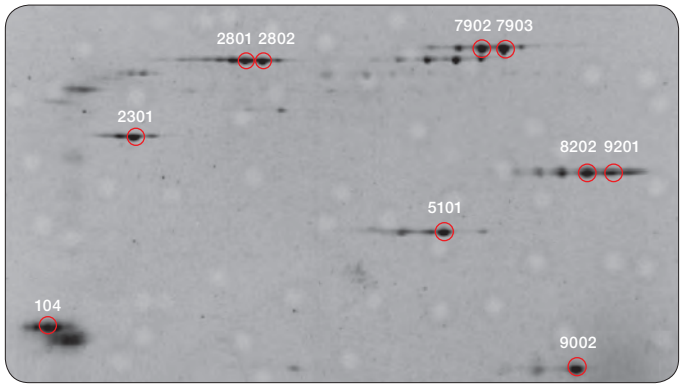


Fig. 1. Images acquired using Molecular Imager VersaDoc imaging system and EXQuest spot cutter of a gel stained with Oriole stain. Protein regions are indicated in **A** and MS sample SSP numbers are indicated in **B**.

Table 1. Summary of staining protocols and results for Oriole and SYPRO Ruby stains.

	SYPRO Ruby Stain		Oriole Stain	
Protein	**% total**	**%CV**	**% total**	**%CV**
Trypsin inhibitor	11.8	9.8	7.6	20.5
Actin	8.1	23.9	7.6	21.3
BSA	8.5	22.7	13.4	13.1
Carbonic anhydrase	8.5	13.2	9.1	15.3
Ovotransferrin	22.4	11.3	24.1	6.2
GADPH	25.8	3.4	25.1	10.3
Myoglobin	14.8	9.3	13.2	5.3
Average CV		13.4		13.1
Processing Step	**SYPRO Ruby Stain**		**Oriole Stain**	
Fix	2 x 30 min		none	
Stain	1 x overnight		1 x 90 min	
Destain/wash	1 x 60 min		none	
Total time	~18 hours		90 min	
Number of processing steps	4		1	
Volume of all reagents/gel	400 ml		100 ml	

Table 2. Comparison of the MALDI-TOF analysis of digested spots. Percent sequence coverage and number of peptides matched following automated search of the Swiss-Prot database.

		SYPRO Ruby Stain		Oriole Stain	
Protein*	**SSP**	**Coverage**	**Peptides**	**Coverage**	**Peptides**
ITRA_SOYBN	104	21	7	35	13
ACTA_BOVIN	2301	35	16	36	15
ALBU_BOVIN	2801	20	10	37	19
ALBU_BOVIN	2802	20	13	57	39
CAH2_BOVIN	5101	53	17	48	21
TRFE_CHICK	7902	20	12	45	37
TRFE_CHICK	7903	26	14	52	43
G3P_RABIT	8202	17	9	18	14
G3P_RABIT	9201	32	15	28	18
MYG_HORSE	9002	54	12	62	15

* Swiss-Prot/UniProtKB protein ID.

stain is its short gel processing time. Gels can be directly stained following electrophoresis. Furthermore, optimal staining is achieved in 90 min and overstaining does not occur. In contrast, the majority of fluorescent gel stains require a separate fixation step prior to staining and rely on an overnight staining step for best results. CBB counterstaining, when needed, leads to additional costs in terms of time and reagents. In practical terms, this means that when the gels stained with Oriole stain are ready for spot cutting, other gels need another day until they are at the same stage.

Spot Cutting and MALDI Analysis

Earlier work on fluorescent stains and their compatibility with MS (Ball and Karuso 2007) provided excellent insight on staining differences using the same sample set as in this study. The levels of protein were kept above the limit of detection for CBB staining in order to facilitate spot cutting from counterstained gels. We chose to excise the same ten spots as described by Ball and Karuso. Spots from two replicate gels were processed individually and the averages were reported. SSP identifiers generated by the PDQuest software were used to track the individual gel plugs through to MS analysis. While we used an EXQuest spot cutter, manual spot cutting (40 spots total) could have been used.

The strong absorbance of Oriole stain in the UV range produces a high-contrast pattern in the EXQuest spot cutter identical to that obtained with an imager. Figure 1A shows a typical gel image obtained on a VersaDoc MP 4000 imager with a 20 sec exposure. Exposures of 30–60 sec on the EXQuest spot cutter are sufficient to produce identical images in terms of contrast and resolution (Figure 1B). Longer exposure time is not an issue as Oriole stain is not affected by photobleaching. When exposed to UV irradiation for 20 min, more than 70% of the peak fluorescence is retained (data not shown).

Both SYPRO Ruby and Oriole stains led to the correct identification (p <0.05 confidence level) of all 10 spots by MALDI-TOF analysis (Table 2). Most spectra were peak rich and free of interfering contaminants. In general, the sequence coverage of the proteins was similar between the two stains except for a few samples. For example, the sequence coverage and number of identified ovotransferrin and BSA peptides derived from gels stained with Oriole stain surpassed those from SYPRO Ruby stained gels despite an equivalent load of proteins on all gels. SYPRO Ruby has been reported to lead to MS results lacking a significant amount of cysteine-containing peptides (Lanne and Panfilov 2005). We examined results obtained from the analysis of BSA (SSP 2801, Table 2) from both samples. When the spectra and peak lists were compared, it was found that 8 of the 10 peptides identified from the SYPRO Ruby gels were common with those from gels stained with Oriole stain, one containing a cysteine residue. Among the 11 peptides that were unique to the Oriole stain sample, 9 contained at least one cysteine residue. Although it will need to be confirmed by independent experiments, these results suggest that Oriole stain does not interact with proteins and peptides in the same manner as SYPRO Ruby stain.

Conclusions

Overall, Oriole fluorescent gel stain has sensitivity, dynamic range, and MS compatibility that is comparable to most commercially available fluorescent gel stains. However, Oriole stain's short staining time and high UV absorbance can save up to one day in a 2-D SDS PAGE and MS analysis workflow.

References

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Lin JF et al. (2008). Stain efficiency and MALDI-TOF MS compatibility of seven visible staining procedures. Anal Bioanal Chem 390, 1765-1773.

Neuhoff V et al. (1988). Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. Electrophoresis 9, 255-262.

White IR et al. (2004). A statistical comparison of silver and SYPRO Ruby staining for proteomic analysis. Electrophoresis 25, 3048-3054.

All cDNA Synthesis Kits Are Not Created Equal: RNaseA Inhibition and Its Effect on qPCR Data

Ribonucleases (RNases) catalyze the degradation of RNA into smaller components, and are ubiquitous in a laboratory environment. While RNases have many essential functions within a cell, they are generally problematic outside the cell acting as a contaminant to researchers working with RNA. Endogenous RNases that co-purify during sample preparation and perspiration from ungloved hands can both contaminate pipet tips, tubes, buffers, water, and surfaces that come in contact with RNA. Researchers combat RNase contamination by using dedicated pipets, aerosol-barrier tips, DEPC-treated water and buffers, and wiping surfaces with RNase-inhibiting agents. Figure 1 shows varying levels of RNase contamination in different preparations of spleen and pancreas RNA. Contaminating RNase in a reverse transcription (RT) reaction can have adverse effects on gene expression analysis. Studies have shown that degraded RNA can cause threshold cycle (C_T) delays in quantitative PCR (qPCR), which in turn can significantly affect overall mRNA quantification. Inaccurate representation of target gene levels can result from using degraded mRNA as a template for cDNA synthesis. The impact can be dramatic, especially when there are small differences in the expression levels between control and treated samples.

Not all cDNA synthesis kits have the same capacity to protect the integrity of RNA. An optimal level of RNase inhibitor is critical for powerful RNase inhibition and efficient reverse transcription. The iScript™ cDNA synthesis kit comes with a 5x iScript reaction mix and iScript reverse transcriptase, which is also blended with a potent RNase inhibitor. Figure 2 shows how the iScript cDNA synthesis kit significantly inhibits RNase activity.

To demonstrate the functional relevance of RNase inhibition in RT-qPCR, an experiment was performed with spiked RNaseA and appropriate controls (Figure 3). There was no C_T delay between cDNA synthesis performed without RNaseA and cDNA synthesis performed with spiked RNaseA (<0.5 C_T difference), showing powerful inhibition of RNaseA. The iScript cDNA synthesis kit can inhibit contaminating RNases or RNases that co-purify with sample preparation, and allow accurate qPCR quantification of target genes. The two-tube format (5x reaction mix and iScript reverse transcriptase) also reduces the chance for RNaseA contamination during setup.

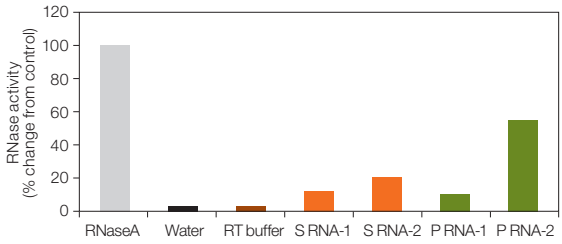


Fig. 1. RNase levels in different RNA samples. RNaseA, water, RT buffer (used to dilute RNaseA), spleen RNA (S RNA-1 and S RNA-2), and pancreas RNA (P RNA-1 and P RNA-2) from two different preparations were assayed for RNaseA activity using a commercially available kit. RNaseA (several fold higher than normally found in contaminated RNA preparations) was used as control to demonstrate varying levels of RNaseA activity in sample preparations.

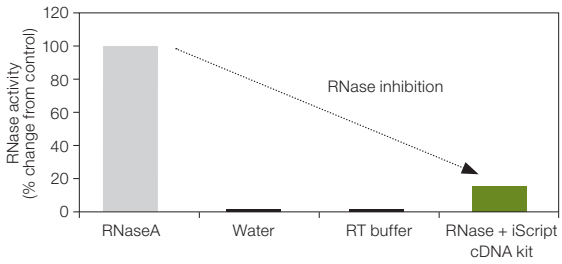


Fig. 2. RNase inhibition with iScript cDNA synthesis kit. RNaseA, water, RT buffer (used to dilute RNaseA), and RNase + iScript cDNA kit were assayed for RNaseA activity using a commercially available kit. RNaseA (several fold higher than normally found in contaminated RNA preparations) was used as control to assess the effect of inhibition by the iScript cDNA kit.

For more information on the effect of RNA degradation on data quality in qPCR, request **bulletin 5452**.

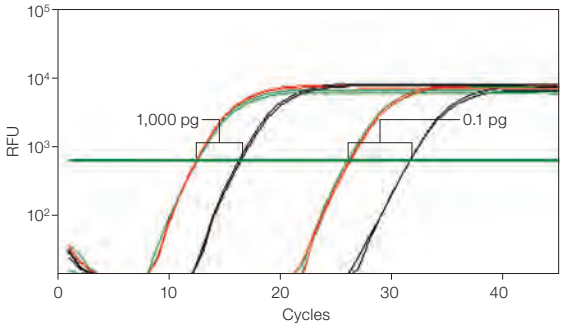


Fig. 3. Functional RT-qPCR assay demonstrating RNaseA inhibition by iScript cDNA synthesis kit. 18S rRNA was amplified using iQ™ SYBR® Green supermix. Reverse transcription (RT) performed with iScript (—); RT performed with spiked RNaseA (—); RT performed with RNaseA but no RNase-inhibitor (—). Two RNA inputs (1,000 and 0.1 pg) were reverse transcribed for all three groups. The cDNA synthesis reaction with RNase but no RNase-inhibitor showed significant C_T delay (3–5), whereas the iScript kit demonstrates powerful inhibition of RNaseA. RFU, relative fluorescence units.

ProteOn™ XPR36 Protein Interaction Array System 2010 Webinar Series

Surface plasmon resonance (SPR) has revolutionized the study of the protein interactions required for the execution and maintenance of complex biological processes. SPR studies have helped to elucidate the roles that intracellular concentration, ionic environment, cofactors, and protein conformation play in maintaining those processes. Just as important, SPR has become a powerful tool for drug development, including the detailed evaluation of drug lead compounds, optimization of affinity protein purification methods, and rapid identification of highly specific monoclonal antibodies with high affinity for the analyte of interest.

The ProteOn XPR36 protein interaction array system from Bio-Rad integrates state-of-the-art microfluidics with a novel optical design to create a unique 6 x 6 interaction array (Figure 1). Up to six ligands can be injected through channels in the vertical direction and up to six analytes can be injected through channels in the horizontal direction, producing 36 data points in a single experiment (Figure 1). This process also creates unique horizontal interspots that have not been exposed to the ligand or any of the reagents used to bind the ligand to the sensor chip. These interspots are used to measure the nonspecific interaction of the analyte with the chip surface, bulk effects, and signal drift.

Last year, Bio-Rad launched its "Become an SPR Expert" webinar series to highlight the SPR research of ProteOn system customers. The webinars included speakers representing pharmaceutical, biotechnology, and government research sectors to demonstrate the wide ranging capabilities of the instrument:

- **Epitope Binning Using the ProteOn XPR36**
  Presented by Yasmina Abdiche, Ph.D, Pfizer Rinat
- **Kinetic Screening of an scFv Antibody Fragment Library Using the ProteOn XPR36 Interaction Array System**
  Pesented by Olan Dolezal, Ph.D,
  CSIRO Molecular and Health Technologies
- **ProteOn XPR36 and Lipoparticle Technology: a Powerful Combination for Screening Antibody Therapeutics Against Membrane Proteins**
  Presented by Sharon Willis, Ph.D., Integral Molecular

Based on the success of the debut series, Bio-Rad is continuing to share customer experiences on the ProteOn system with a new lineup of speakers for 2010. All 2009 and 2010 webinars can be downloaded at **https://biorad.box.net/proteonxpr36webinars**.

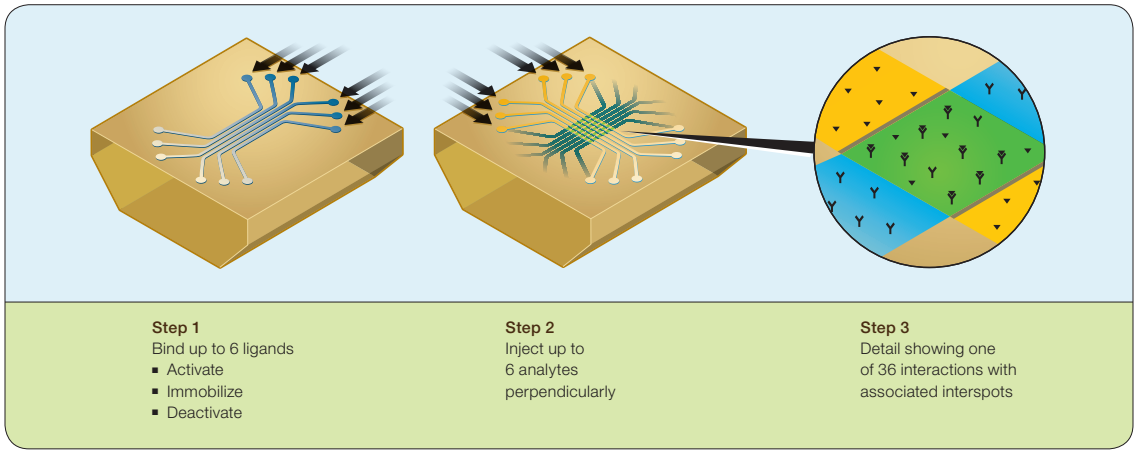


Fig. 1. Generation of the 6 x 6 ligand-analyte interaction array with unique interspot referencing.

2010 Webinar Topics and Presenters

April 19, 2010

Presented by
Torbjörn Pettersson, PhD
Magnus Branden, PhD
Layerlab, Sweden

Novel Liposome Immobilization Technology for Biosensors

This webinar provides an introduction to the novel liposome immobilization technology available from Layerlab. Dr Torbjörn Pettersson and Dr Magnus Branden discuss immobilization protocols for liposomes and lipoparticles as well as novel methods for the screening of drug candidates against malaria using SPR. “Since membrane proteins are not water soluble, their functionality can be difficult to analyze. We demonstrate how to screen membrane protein functionality in a native-like environment on the biosensor platform,” explains Pettersson.

May 4, 2010

Presented by
John Kulman, PhD
Puget Sound Blood Center
Seattle, Washington

A Calcium-Dependent Immunocapture Strategy for Enhanced Throughput SPR

The applicability and throughput capacity of SPR–based assays are often limited by the time and expense involved in generating high-quality ligands, as well as by difficulties in ensuring their integrity over multiple regeneration cycles. Dr John Kulman has developed a method for the reversible on-chip capture of ligands from the complex medium of transiently transfected mammalian cells. This approach determines kinetic and thermodynamic parameters governing antibody-antigen interactions with unsurpassed precision and speed — for a fraction of the cost and effort of conventional approaches. “I hope viewers will gain an awareness that SPR data are only as good as starting reagents, an understanding of how calcium-dependent immunocapture can be used to generate high-quality data with minimal time and resources, and an appreciation for how SPR can be used to perform comprehensive thermodynamic analysis of antibody-antigen interactions,” explains Kulman.

June 8, 2010

Presented by
Tsafrir Bravman, PhD
Bio-Rad Laboratories, Inc.
Haifa, Israel

The Study of Small Molecule-Protein Kinase Interactions Using Multiplexed SPR — Speeding Drug Discovery

The drug discovery workflow includes a large screening component in which high sensitivity, reproducible binding measurements are an absolute requirement. Binding assays must be developed quickly to respond to the changing needs of the development process while still maintaining throughput. Dr Tsafrir Bravman presents data from studies investigating the interaction of small molecule kinase inhibitors against important kinase targets to demonstrate how the ProteOn XPR36 system can be used to accelerate drug discovery. “I hope viewers gain insight into the capabilities and advantages of SPR label-free measurements, and the power to perform these measurements in an efficient, rapid, and robust way,” says Bravman.

September 16, 2010

Presented by
Gideon Schreiber, PhD
Weizmann Institute
Israel

Pushing the ProteOn XPR36 System to its Limits: From Protein-Protein Docking to Nanoparticle Interactions

During the last five years, the Weizmann Institute has been using the ProteOn XPR36 system across a wide range of applications, from providing experimental data driving protein-protein docking to protein engineering and design, as well as for learning how proteins and peptides interact with gold nanoparticles. Dr Gideon Schreiber presents a series of tools and research protocols for measuring many types of interactions in a multiplex design, taking advantage of the unique 6 x 6 architecture of this instrument. “I hope that sharing the knowledge we have cultivated over the past 5 years will help other researchers most effectively plan their experiments,” says Schreiber.

To download the 2009 and 2010 ProteOn system webinars, please go to **https://biorad.box.net/proteonxpr36webinars**.

Unlocking the Potential of Stem Cell Research Using the EpiQ™ Chromatin Analysis Kit

Dr David Schaffer
University of California
at Berkeley
Professor of
Chemical Engineering,
Bioengineering,
and Neuroscience
Codirector Berkeley
Stem Cell Center

Introduction

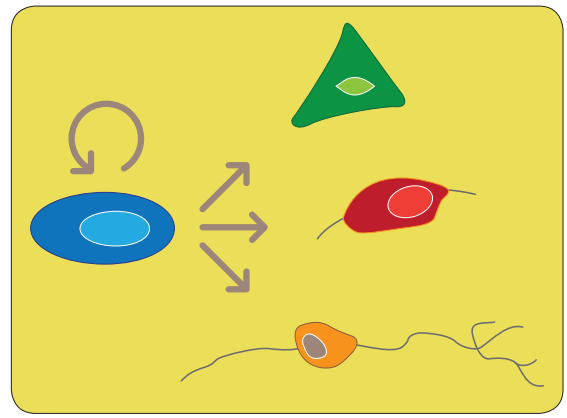
Bedtime stories in Dr David Schaffer's childhood home were often not composed of standard fairy tales. With both parents in careers as biomedical researchers (his mother in drug development and clinical trials for a major pharmaceutical company and his father, in cardiovascular research and a pharmacology professor) and, much of the conversation as far back as Schaffer can remember centered on biology and science. "I remember being 5 years old and sitting on my father's lap, while he was teaching me the names of microorganisms," says Schaffer. In many ways, a life dedicated to solving biological problems seemed predetermined, but Schaffer did deviate slightly from the examples and influences of his parents and initially concentrated on the engineering aspects of the field.

Schaffer completed his undergraduate work at Stanford University in chemical engineering and graduate work in chemical engineering at MIT. His postdoctoral work was in the laboratory of Dr Fred Gage, a neurobiologist at the Salk Institute for Biological Studies. "For 2 years, I was the only engineer at the Salk Institute, and had immersed myself in the rich world of biology in a lab that had been making some paradigm-shifting discoveries in the field of neural stem cells and understanding how the adult brain continues to add neurons," says Schaffer. It was during this pivotal period that Schaffer became fascinated with applying engineering approaches to the study of problems in stem cell biology.

Pioneering Stem Cell Research

Upon completion of his postdoc work, Schaffer accepted an assistant professor position at UC Berkeley and for the past 10 years the main focus of his laboratory has been developing engineering systems that provide insight into the signals that control cell function. Schaffer credits California's Proposition 71 (a voter initiative passed in 2004 that created a framework to legalize, regulate, and fund stem cell research in the state) as having "provided us with incredibly valuable resources to be able to research stem cells — California is unique in that respect."

Researchers in the Schaffer laboratory are primarily engaged in understanding the transcriptional regulation of the key genes involved in stem cell functions. "There are several key genes that are absolutely necessary for a stem cell to function as a stem cell," explains Schaffer. "In the case of human embryonic stem cells, pluripotency genes must be on and functioning. So examining the state of those genes — of the DNA inside the cell — is critical for our work."



Stem cells. Stem sells are defined by self-renewal and differentiation.

Schaffer describes the two main applications of stem cell research as using stem cells for therapeutic purposes (cell replacement therapy and regenerative medicine) and gaining insight into the mechanisms of disease (creating model systems of a diseased mature cell type). However, there have been major challenges to overcome before this potential can be realized. "There is no situation in which a grown adult has access to embryonic stem cells that are a perfect genetic match for them," explains Schaffer. "So if you'd like to use embryonic stem cells for therapeutic purposes, you might have tissue rejection issues." And as far as using stem cells to create diseased cells (for example, neurons in the case of Alzheimer disease), "The problem with using human embryonic stem cells is that almost by definition — the fact that you've made a stem cell line out of it — you'll never have the opportunity to know if that person, that embryo, would have contracted Alzheimer disease 70 years into the future."

The Power of Reprogramming

The recent advent of "induced pluripotency" or "reprogramming" has made it possible for researchers to take any cell in the body and essentially turn back the clock creating an embryonic-like cell. So now not only can the clock be moved forward to create a more mature stem cell, neuron, or a B cell from a pluripotent stem cell, but researchers can take mature cells and introduce certain genes and turn the clock backwards — reprogramming the cell into an embryonic state. "What you can do then," explains Schaffer, "is take a stem cell, B cell, or keratinocyte from a 70-year-old person who has Alzheimer disease, turn back the clock on it, and then turn the clock forward again and basically use it as an infinitely renewable resource to create as many diseased neurons as you'd like." This capability allows researchers to study the mechanisms of disease as well as the effects of potential drugs on disease. Further, this reprogramming technology virtually eliminates limited resource issues that have previously stymied rapid progress in the field.

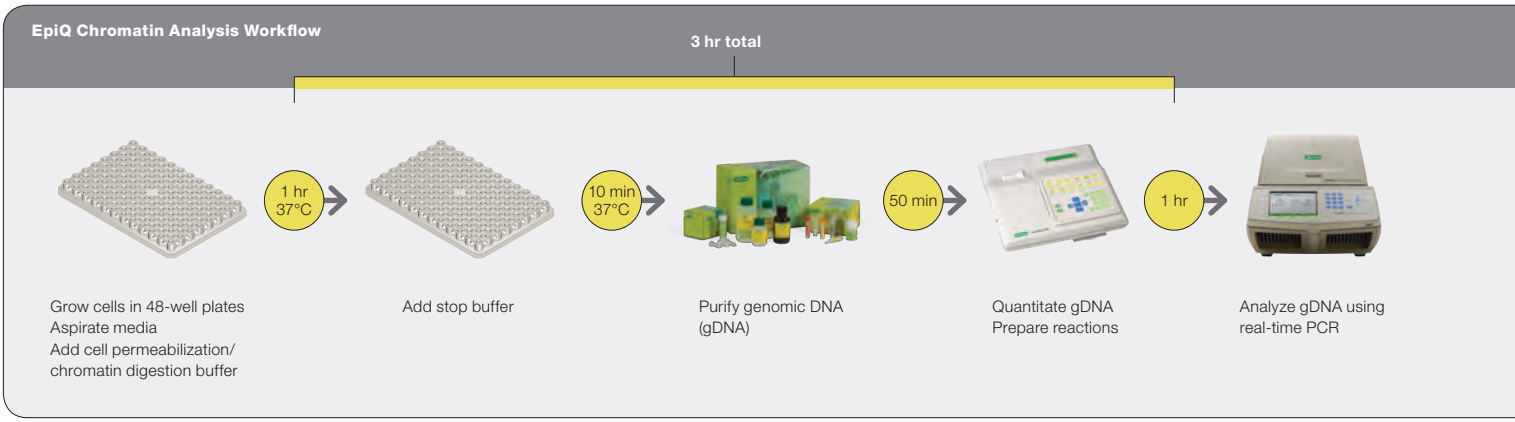
But the process of reprogramming can be laborious and inefficient. To study the mechanisms of cellular reprogramming and gain insight into the state of genes critical for stem cell function — whether the genes are on or off and how close they are to being in an embryonic state — has required methods like methylation analysis and chromatin immunoprecipitation. But while these tests provide detailed molecular readouts and valuable information about the gene(s) involved, they require significant time to perform; there has not been a quick and easy way for researchers to check gene status mid-experiment. In addition the reprogramming process itself is inefficient with experiments typically requiring 10–20 attempts that can all have varying results. That's why the Schaffer laboratory was interested in testing Bio-Rad's new EpiQ chromatin analysis kit.

Rapid Insight into Gene States

Epigenetic processes control gene expression by altering chromatin structure. The chromatin analysis kit assesses chromatin structure in cultured cells, providing insight into gene expression in an easy-to-use method that can be performed the same day cells are harvested in approximately 3 hours. "What the kit has enabled us to do is study the state of genes during the reprogramming process in a very rapid and efficient way," explains Schaffer. "We are now able to obtain information about whether reprogramming is proceeding or how it is proceeding ten times as fast as other methods we'd been using, plus the assay tells us whether or not it's even worthwhile going through the trouble to perform deeper analyses." Unlike other tools that don't provide insight into gene states until all experimentation has been completed, the kit has enabled rapid examination of the state of the genes that must be turned on during reprogramming and provides researchers with early views of what does go wrong or what can go right during the reprogramming process. "It tells us how quickly those genes start turning on and whether or not their lack of turn on at an early stage is diagnostic for a low efficiency of reprogramming at the end," says Schaffer.

The Schaffer laboratory is also using the kit to study HIV latency — attempting to understand how the HIV gene turns on and off once it has inserted itself into the genome of a cell. "We're interested in understanding the state of the HIV gene in a genome and how its structure — its chromatin structure, its DNA structure — how this relates to the dynamics by which the virus turns on or stays off," Schaffer explains.

Clearly, ongoing research in the Schaffer laboratory has the potential to unlock the mysteries surrounding some of the most degenerative and life-threatening diseases of our time. And the EpiQ chromatin analysis kit is a novel tool that is helping to rapidly and efficiently continue to move this research forward.



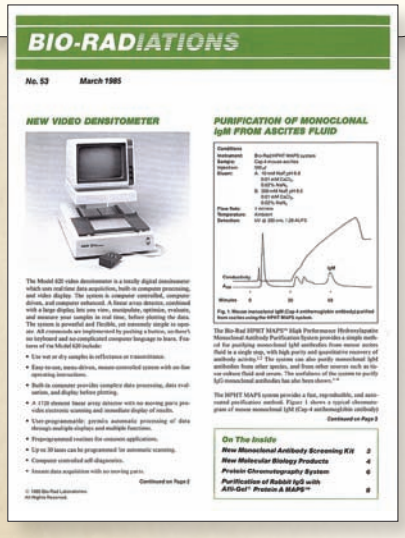
BioRadiations 1985: Video Densitometer Represents Cutting-Edge of Imaging Technology

In this column, we look back at previously published issues of *BioRadiations* to see how tools and applications have changed since Bio-Rad first began publishing the journal in 1965.

Then!

Built-in Computer for Complete Data Manipulation on the Screen

The densitometer lets you process, optimize, evaluate, and view your data on a large video screen before you plot it. You can even measure peak heights and integrals directly on the screen. When you are ready to plot, the computer can control the plotting automatically, with no operator involvement. You can view, evaluate, measure, and plot either a single lane or multiple lanes of data.


BIO-RADIATIONS

No. 53 March 1985

NEW VIDEO DENSITOMETER

PURIFICATION OF MONOCLONAL IgM FROM ASCITES FLUID

On The Inside

New Monoclonal Antibody Screening Kit 3

New Molecular Biology Products 4

Protein Chromatography System 6

Purification of Rabbit IgG with Affi-Gel® Protein A MAPS™ 8

Now!

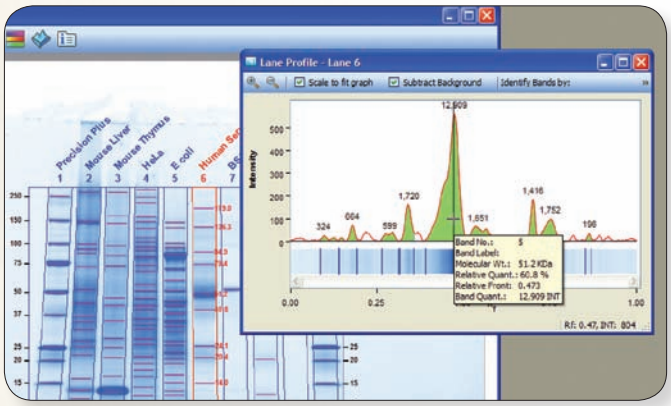
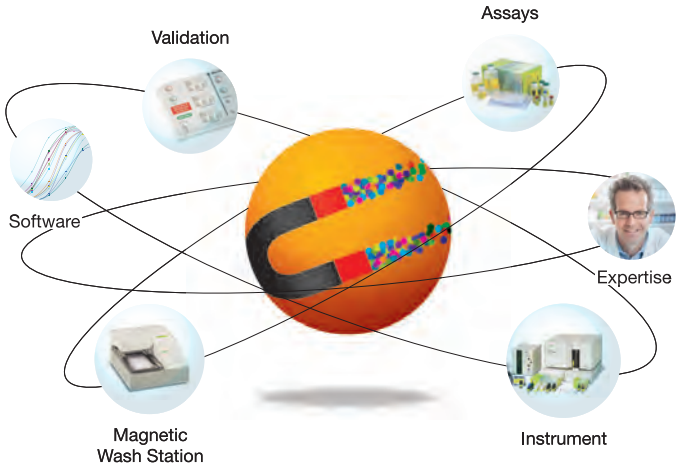


Image Lab™ software.

In 1985, software automation of data capture, processing, and analysis was just beginning across many areas of scientific research — including gel electrophoresis. At that time, two-dimensional video densitometry introduced the ability to perform spot matching and information archival at the benchtop versus more complex and bulky computer workstations. Today, Bio-Rad remains a leader in the field of gel imaging and analysis with a spectrum of benchtop systems (see this issue's feature article, page 16) that continue to maximize image capture and analysis capabilities while minimizing complexity and lab space required.

"I get the flexibility I need with **multiplexcellent** assays."



Magnetic bead–based multiplex assays.

Bio-Plex Pro™ Assays now feature revolutionary magnetic assay technology, demonstrating Bio-Rad's commitment to enhancing your experience through innovative improvements of the Bio-Plex® system. Using Bio-Plex magnetic bead–based assays, you get increased assay reproducibility with the flexibility to choose between filtration and magnetic separation.

From cytokines to disease-specific analytes, intuitive software to integrated validation solutions, Bio-Plex provides you with the tools for complete confidence in your results. Visit us at **www.bio-rad.com/ad/bio-plex** to learn more.

Research. Together.

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- Fit cell counting into your schedule with easy-to-use software and results in less than 30 seconds
- Trust your counts with an innovative autofocus technology that removes the subjectivity of manual focusing
- Get faster and more reproducible downstream results using accurate and consistent cell counts

Speed up your cell counting — and accelerate your research. Visit **www.bio-rad.com/ad/countelligent** for more information.

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