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In this issue:

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Aiding Advances in Cardiomyopathy Research

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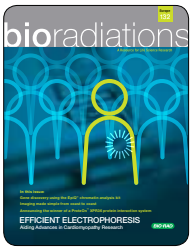
On the cover:
Conceptual illustration by Diana Kollanyi.



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bioradiations

TO OUR READERS

The electrophoresis and blotting workflow was established in the 1970s to enable researchers to begin to characterize the abundance of genetic information made possible through advances in DNA technologies. Since this inception, Bio-Rad has played an important role in developing tools to streamline processes and optimize results obtainable in this routine science workflow. Most recently, we have developed long shelf life TGX™ gels, the Gel Doc™ EZ one-button imaging system, and the Trans-Blot® Turbo™ rapid transfer blotting system — each designed to significantly reduce the time required to perform the running, imaging, or transferring of gels. This issue's cover story traces the experience of testing the Trans-Blot Turbo system in the Gomes Lab at the University of California, Davis, and demonstrates how improvements in electrophoresis technologies have impacted the ability of these researchers to resolve questions related to heart disease.

COVER STORY

DEPARTMENTS

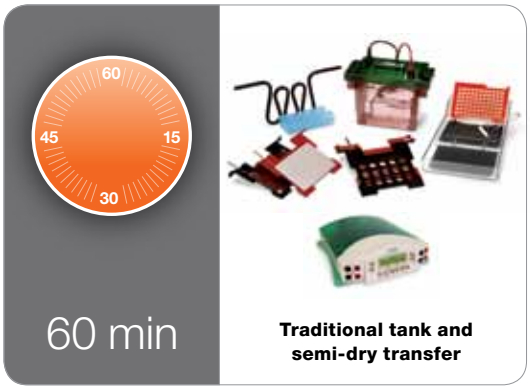
TECHNICAL REPORTS

Getting to the Finish Line Faster

Introducing the Trans-Blot® Turbo™ Transfer System

Western blotting is a key step in the process of protein identification. While this step is critical in the overall electrophoresis workflow, there has been little innovation in blotting over the past 30 years; the blotting step remains cumbersome and time-consuming — often requiring several hours to complete.

Bio-Rad's Trans-Blot Turbo transfer system is designed to accelerate the protein transfer process without compromising quality. Western transfer is now possible in as little as 3 minutes.



Key features include:

- **Superior performance** — both high– and low–molecular weight proteins are efficiently transferred in 7 min using Trans-Blot Turbo transfer packs
- **TGX™ turbo transfers** — proteins are transferred from a MiniPROTEAN® TGX™ gel in as little as 3 min
- **Higher throughput** — transfer up to 4 mini or 2 midi gels in 1 run, twice the number of blots as our leading competitor in the same amount of time
- **System flexibility** — this high-throughput blotting system can perform both turbo transfers with our Trans-Blot Turbo transfer packs and traditional semi-dry transfers with conventional membranes, filter papers, and buffers
- **Convenient transfer packs** — provide membranes and filter paper that are precut, prewetted, and ready to use in a single pack format
- **Intuitive interface** — designed for ease of use with optimized preloaded protocols and the ability to save/recall user-defined protocols

Trans-Blot Turbo system throughput capabilities.

Throughput (per run)	1–4 mini gels or 2 midi gels	
Transfer time	3 min (Mini-PROTEAN TGX gels only)	Transfers up to 100 kD
	7–10 min	Transfers up to 400 kD
Transfer efficiency	Linear from 5–200 kD	
Transfer modes	Traditional semi-dry transfer Rapid transfer	

Ordering Information

Catalog #	Description
170-4155	Trans-Blot Turbo Starter Kit, includes Trans-Blot Turbo system, Precision Plus Protein WesternC standards, mixed transfer pack kit
170-4152	Trans-Blot Turbo Base, includes system without transfer cassettes
170-4151	Trans-Blot Turbo Cassette, includes 1 transfer cassette
170-4156	Trans-Blot Turbo Transfer Pack, 0.2 µm PVDF, 7 x 8.5 cm, 10 pack
170-4157	Trans-Blot Turbo Transfer Pack, 0.2 µm PVDF, 8.5 x 13.5 cm, 10 pack
170-4158	Trans-Blot Turbo Transfer Pack, 0.2 µm nitrocellulose, 7 x 8.5 cm, 10 pack
170-4159	Trans-Blot Turbo Transfer Pack, 0.2 µm nitrocellulose, 8.5 x 13.5 cm, 10 pack

For more information, visit **www.turboblotter.com**, or request **Bulletin 6039A**.

Bio-Plex Pro™ Cytokine and Diabetes Assays

All multiplex immunoassays offer:

- **Quality** — designed for enhanced assay sensitivity and working ranges
- **Robustness** — validated in serum, plasma, tissue culture, urine*, and milk* matrices
- **Increased productivity** — measure multiple analytes in a single reaction in as little as 3 hr (mouse and rat assays) or 5 hr (TGF-β assays)
- **Option to use a magnetic wash station** — take advantage of the magnetic workflow for simplified assay preparation and achieve consistent, improved reproducibility (also compatible with the vacuum wash station)
- **Flexible ordering options** — customize your assays by choosing the analytes of interest and either a flat-bottom plate for magnetic washing or a filter plate for vacuum washing. Choose either a premixed panel, the singleplex analytes of interest, or customized Bio-Plex® x-Plex™ or Express assays; visit **www.bio-rad.com/assaybuilder** to learn more about custom assays
- **Convenient, space-saving, all-in-one format** — includes the assay (tube of magnetic beads coupled with capture antibodies and tube of detection antibodies), reagent kit, and a vial of standards

For a complete listing or to obtain ordering information, go to **www.bio-rad.com/bio-plex**.

Bio-Plex Pro™ Mouse Cytokine Th17 Assays

The Bio-Plex Pro mouse cytokine panel detects analytes implicated in Th17 cell lineage and its pathway. Th17 plays a role in host defense against extracellular pathogens. Aberrant regulation of Th17 cells are implicated in the pathogenesis of multiple inflammatory and autoimmune disorders.

Bio-Plex Pro™ Rat Cytokine Assays

The Bio-Plex Pro rat cytokine assays are an expansion upon our previous rat cytokine assays. They are magnetic bead-based multiplex assays that detect multiple cytokines and are compatible with either magnetic- or vacuum-based washing methods. This panel is compatible with the new Bio-Plex Pro rat diabetes assay for an expanded biomarker profile; please note that the current Bio-Plex rat cytokine 9-plex panel (and associated reagents and diluents) is being replaced by this assay panel. Neither the reagent kits nor the instruction manual are interchangeable due to many differences, including new bead regions, protocol, and incubation times.

*TGF-β assays only.

Summary of Ordering Options

			Custom Assay Options via Assay Builder	
Bio-Plex Pro Assay	**Pre-mixed/fixed panel**	**Singleplex Sets***	**Express****	**x-Plex*****
TGF-β	•	•	—	—
Mouse cytokine Th17	•	•	•	•
Rat cytokine	•	•	•	•
Rat diabetes	—	•	•	—

* Singleplex sets comprise a vial of beads coupled to capture antibodies and a vial of detection antibodies; require a reagent kit and standards to perform an assay.

** Express assays comprise singleplex sets for the analytes of interest plus a reagent kit and standards; require the user to mix analytes at the bench.

*** x-Plex assays comprise the multiplexed analytes of interest plus a reagent kit and standards. Multiplexed analytes of interest are quality controlled by Bio-Rad; this is a premium service and requires longer delivery times.

Bio-Plex Pro™ Rat Diabetes Assays

The Bio-Plex Pro rat diabetes assays are magnetic bead–based multiplex assays that detect diabetes and obesity biomarkers in a single experiment, using as little as 12.5 µl of sample per well. These assays offer enhanced assay performance and compatibility with Bio-Plex Pro wash stations. Mix rat cytokine and diabetes assays for an expanded biomarker profile.

Bio-Plex Pro™ TGF-β Assays

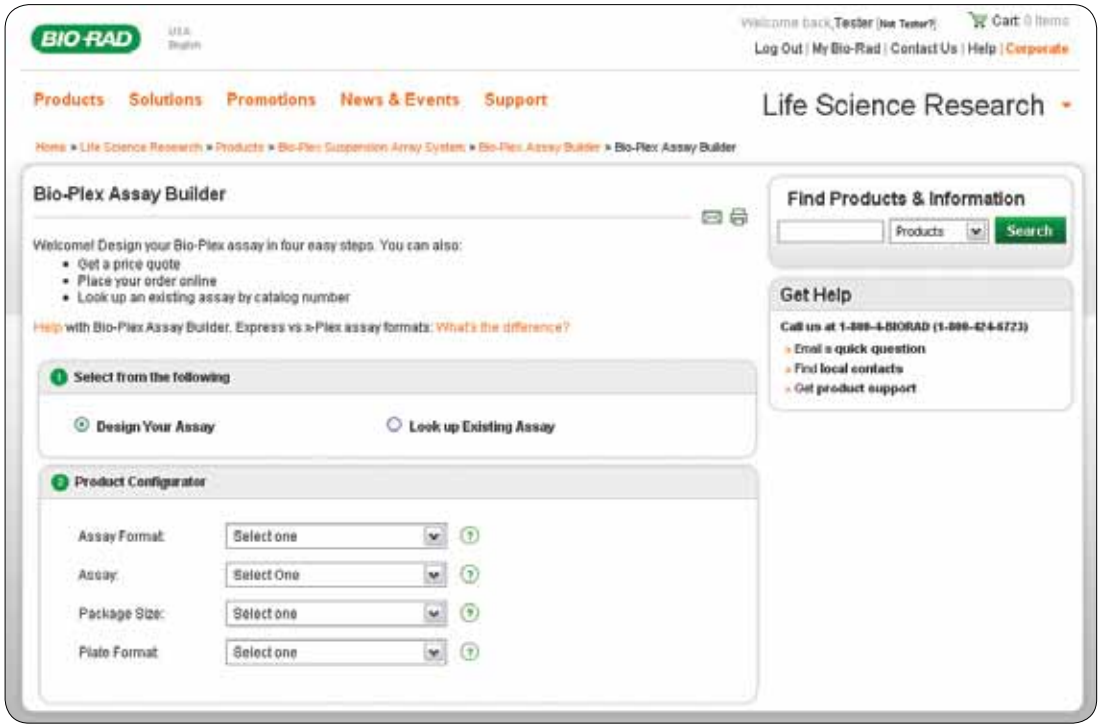
The TGF-β family of proteins plays an important role in cellular functions such as proliferation, differentiation, migration, and apoptosis. There are three known isoforms of TGF-β with functions in normal physiology and in disease states such as cancer, diabetes, and cardiovascular illness. Bio-Plex Pro TGF-β assays are magnetic bead-based multiplex assays designed to measure TGF-β1, TGF-β2, and TGF-β3 in human, mouse, and rat sample matrices such as serum plasma, urine, and milk. These assays offer enhanced performance and compatibility with Bio-Plex Pro wash stations.

Bio-Plex® Assay Builder* — Now it is Even Easier to Customize Assays

The Bio-Plex assay builder offers customized assays for increased flexibility. Try the online tool at **www.bio-rad.com/assaybuilder** and:

- Create a custom assay in 4 easy steps
- Immediately place orders online or request a quote
- Save your favorites
- Select the assay plate you need for your specific wash method — filter plate for vacuum filtration or a flat-bottom plate for magnetic separation
- Easily navigate with online help

* The updated assay builder is currently available in North America and will become available in selected countries during 2011.



Bio-Plex assay builder simplifies assay ordering.

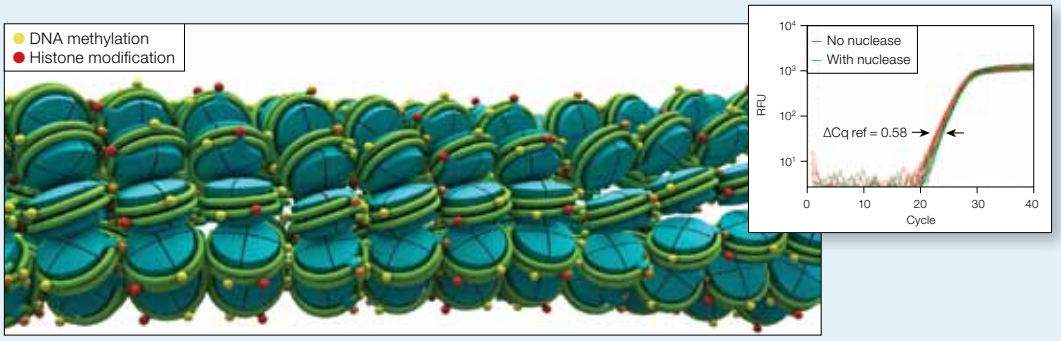
For a complete listing or to obtain ordering information, go to **www.bio-rad.com/bio-plex**.

EpiQ™ Chromatin Analysis Kit

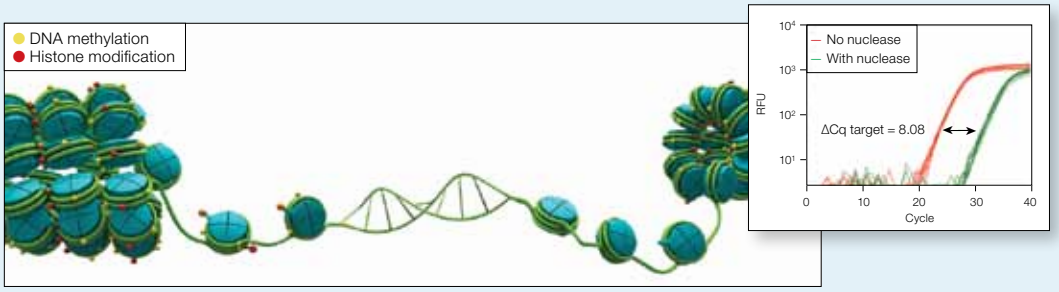
The EpiQ chromatin analysis kit is a real-time PCR assay for the quantitative assessment of chromatin structure and nuclease accessibility in cultured cells. By combining in situ chromatin digestion, genomic DNA purification, and real-time PCR, the chromatin state for several gene promoters can be studied simultaneously. The EpiQ kit helps quantify the impact of epigenetic events, such as DNA methylation and histone modification, on gene expression regulation through chromatin state changes.

- Rapid assessment of chromatin structure in cultured cells (time to results <6 hr)
- Few cells (as little as 5 x 10^4) required to perform analysis
- Novel assay generates quantitative chromatin structure information with strong correlation to gene expression levels

Heterochromatin. These regions of chromatin are inaccessible to transcriptional machinery and are considered transcriptionally silent. In the EpiQ assay, heterochromatin is also inaccessible to the nuclease and the genomic DNA is protected from digestion, making it available for qPCR. This results in a minimal Cq shift relative to the control untreated sample (no nuclease).



Euchromatin. These regions of chromatin are generally accessible to transcriptional machinery and are considered transcriptionally competent. In the EpiQ assay, euchromatin is also accessible to the nuclease and the genomic DNA is susceptible to digestion, making it unavailable for qPCR. This results in a large Cq shift relative to the control untreated sample (no nuclease).



Ordering Information

Catalog #	Description
172-5400	EpiQ Chromatin Analysis Kit, 50 preparations, contains components for chromatin digestion, analysis, and genomic DNA purification, and 2x real-time PCR mix for 500 x 20 µl reactions
172-5401	EpiQ Chromatin Analysis Kit, 100 preparations, 1,000 x 20 µl reactions
172-5402	EpiQ Chromatin Preparation Kit, 50 preparations, contains components for chromatin digestion, analysis, and genomic DNA purification prior to real-time PCR analysis
172-5403	EpiQ Chromatin Preparation Kit, 100 preparations
172-5404	EpiQ Chromatin SYBR Green Supermix, 5 ml, 2x real-time PCR mix, contains dNTPs, iTaq DNA polymerase, $MgCl_2$, SYBR Green I, ROX passive reference dye, fluorescein, stabilizers, for 500 x 20 µl reactions
172-5405	EpiQ Chromatin SYBR Green Supermix, 10 ml, for 1,000 x 20 µl reactions

iScript™ Reverse Transcription Supermix for RT-qPCR

The iScript reverse transcription supermix for RT-qPCR is a simple, fast, and sensitive first-strand cDNA synthesis kit for gene expression analysis using real-time qPCR. In one tube, the preblended 5x supermix contains all necessary components (except RNA template) for reverse transcription.

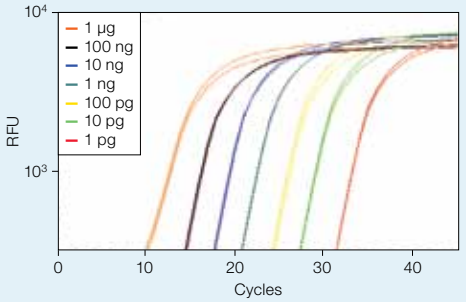
- **Increase reproducibility of reverse transcription and reduce errors during setup** — 1-tube format for simple and fast setup and reduced pipetting variability
- **Detect low-level target genes and conserve RNA during gene expression analysis** — broad linear dynamic range of total input RNA (1 µg–1 pg) with a highly efficient RNase H+ MMLV reverse transcriptase
- **Use with dilute RNA samples** — 5x concentration allows greater volume of RNA sample in a 20 µl cDNA synthesis reaction
- **Eliminate freeze/thaw cycles** — liquid format at –20°C shortens overall processing time and maximizes stability
- **Validate purity of input RNA and gene expression results** — optional no-RT controls for detecting genomic DNA contamination
- **Obtain accurate results** — potent blend of RNase inhibitors prevents RNA degradation during reaction setup and reverse transcription
- **Increase primer design flexibility and prevent 5' and 3' bias** — optimum blend of oligo(dT) and random primers in the 5x supermix for complete RNA coverage
- **Complete cDNA synthesis and qPCR the same day** — fast 40 min cDNA synthesis protocol

Ordering Information

Catalog #	Description
170-8840	iScript Reverse Transcription Supermix for RT-qPCR, 25 x 20 µl reactions, 100 µl of 5x supermix contains reverse transcriptase, RNase inhibitor, dNTPs, primers, $MgCl_2$, stabilizers; 10 reactions of no-RT control supermix
170-8841	iScript Reverse Transcription Supermix for RT-qPCR, 100 x 20 µl reactions

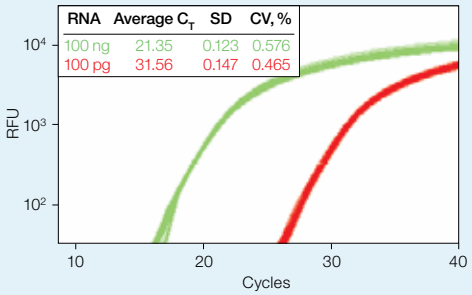
For more information, visit **www.bio-rad.com/iscript**, or request **Bulletin 6031**.

Broad Linear Dynamic Range



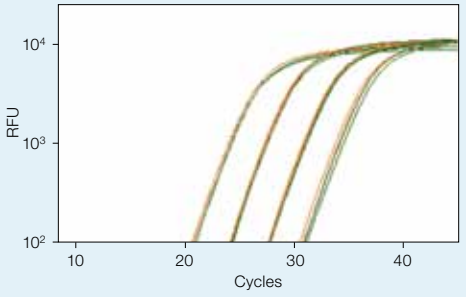
The iScript reverse transcription supermix for RT-qPCR efficiently reverse transcribes RNA over a broad linear dynamic range for reliable gene expression analysis data. Different amounts of HeLa cell RNA (amounts shown in inset) were reverse transcribed and one-tenth of resulting cDNA was used as template to amplify the β-actin gene (~90 bp) in 20 µl qPCR reactions with iQ™ SYBR® Green supermix. Standard curve r^2 = 0.999, efficiency = 99.7%, slope = –3.33. RFU, relative fluorescence units.

Data Reproducibility



Excellent data reproducibility. *PGK-1* mRNA (~160 bp), a gene that encodes a glycolytic enzyme, was quantified using iScript reverse transcription supermix for RT-qPCR both with 100 ng (■) and 100 pg (■) of input RNA. For each input RNA, 48 individual RT reactions were performed and one-tenth of resulting cDNA was used in the qPCR reaction with SsoFast™ probes supermix. The gene expression analysis data show excellent reproducibility both with high and low levels of input target mRNA. The ~10 threshold cycle (C_T) difference for the 1,000-fold dilution of RNA (100 ng–100 pg) demonstrates good reverse transcription efficiencies across different input RNAs. RFU, relative fluorescence units.

Unbiased Representation of 5' and 3' Regions



Unbiased representation of 5' and 3' regions of target genes. Reverse transcription of 100, 10, 1, and 0.1 ng input RNA was performed with iScript reverse transcription supermix for RT-qPCR. Primer pairs were designed at 5' (■, ~60 bp) and 3' (■, ~70 bp) ends of the *MAP* gene and qPCR was performed with one-tenth of input cDNA using iQ™ SYBR® Green supermix. There were no significant differences (<0.5 C_T difference) between the two primer pairs, which demonstrates unbiased representation of 5' and 3' regions. RFU, relative fluorescence units.

CFX Manager™ Software, Version 2.0

CFX Manager software, version 2.0, updates the industry-leading, feature-rich software that provides experiment setup and data analysis capabilities for the CFX96™, CFX384™, and MiniOpticon™ real-time PCR detection systems.

Key features and benefits:

- **Get started quickly** — intuitive navigation and startup wizard
- **Stay organized** — reserve instruments using the Scheduler so they will be available to run your experiments
- **Streamline experiment setup** — rapidly prepare reactions using the Master Mix Calculator
- **Analyze results when and where you want** — email notification with an attached data file is sent when a run is finished
- **Analyze data faster** — easily visualize all run data at once with Custom Data View and export only the data you need with Custom Data Export
- **Perform normalized gene expression** — advanced analysis tools using multiple reference genes and individual reaction efficiencies
- **Run on multiple operating systems** — compatible with Windows XP, Windows Vista, and Windows 7

Specifications

System	Minimum	Recommended
Operating system	Windows XP Professional SP2, Windows Vista Home Premium, Windows 7 Home Premium	Windows XP Professional SP2 and above, Windows 7 Professional
Drive	CD-ROM	CD-RW
Hard drive	10 GB	20 GB
Processor speed	1 GHz	2 GHz
RAM	1 GB (2 GB for Windows Vista)	2 GB
Screen resolution	1,024 x 768 with true-color mode	1,280 x 1,024 with true-color mode
USB port	2.0 Hi-Speed	2.0 Hi-Speed
Internet browser	Internet Explorer	Internet Explorer
Software	—	Microsoft Office Suite

Ordering Information

Catalog #	Description
184-5000	CFX Manager Software, version 2.0, experiment setup and data analysis software for CFX96 and CFX384 real-time PCR detection systems, includes installation CD, quick guides, manual
184-5003	CFX Manager Software, MiniOpticon, version 2.0, experiment setup and data analysis software for MiniOpticon real-time PCR detection system, includes installation CD, quick guides, manual

ProteOn™ XPR36 Protein Interaction Array System

ProteOn Manager™ Software, Version 3.0

ProteOn Manager software is an intuitive, workflow-oriented software package that coordinates instrument control, experiment setup, and data collection and analysis. Version 3.0 software offers increased performance, faster time to results, and easier sample handling and data management.

Features include:

- Advanced report point functionality with flexible report point table creation and manipulation
- Enhanced import and export functionality of data and reports
- Easier and faster protocol creation and sample import/export
- Optimized protocol and analysis reports
- Separate off-rate calculations

Ordering Information

Catalog #	Description
176-0200	ProteOn Manager Software, version 3.0, 1-user license, includes 1 HASP key
176-0210	ProteOn Manager Software, Security Edition, version 3.0, allows U.S. FDA 21 CFR Part 11 compliance, 1-user license, includes 1 HASP key

For more information, request **Bulletin 5627**.

ProteOn™ HTG Sensor Chip

The ProteOn HTG sensor chip features a novel multi-NTA complex for improved capturing of His-tagged proteins. The multi-NTA complexes are attached to a polymer matrix layer and are activated by injection of Ni^{+2} ions for capturing of His-tagged proteins/ligands. Benefits include:

- Full compatibility with the ProteOn XPR36 protein interaction array system
- Improved binding capacity and stability due to the novel Tris-NTA formulation
- Reduced decay of ligand over time — assures reliable results and no cumbersome software corrections needed
- Capture of His-tagged proteins directly from crude media, reducing work-up labor and required sample volumes
- Enhanced efficiency and reduced costs due to surface regeneration ability, providing many ligand-analyte interaction studies on the same chip (typically >10 surface regeneration cycles are possible)

- Surface regeneration combined with One-shot Kinetics™ technique yields exceptionally high throughput

Ordering Information

Catalog #	Description
176-2500	ProteOn HTG Capturing Kit, includes 1 ProteOn HTG Sensor Chip and 1 ProteOn HTG Reagent Kit
176-5031	ProteOn HTG Sensor Chip, includes 1 sensor chip
176-2510	ProteOn HTG Reagent Kit, includes sufficient reagents for >80 activation and regeneration cycles

For more information, request **Bulletin 5404**.

Application of UNOsphere SUPrA™ Media, a New Protein A Affinity Chromatographic Support, for Both Laboratory-Scale and Industrial Antibody Purification

Joseph Siino[1], Xuemei He[1], Wenqiang Tang[2], Dennis C. Yee[1], Mark Snyder[1], Sherif Hanala[1], and Wei Liu[1]
[1]Bio-Rad Laboratories, Inc., 6000 James Watson Drive, Hercules, CA 94547 USA, [2]Institute of Biological Sciences, He Bei Normal University, Shijiazhuang, He Bei 050016

Introduction

Protein A affinity chromatography is a widely used approach for immunoglobulin purification. The relative low cost and high stability of the Protein A media compared to other options for IgG purification make them the most popular choice for antibody purification both at laboratory and industrial scales (Hober et al. 2007).

Bio-Rad's UNOsphere SUPrA™ affinity media is a recombinant Protein A resin developed for a wide range of research, development and manufacturing applications where high binding capacity is required. UNOsphere SUPrA affinity media is built on the proven UNOsphere macroporous polymeric matrix. The media has a binding capacity of 25 to 30 mg/ml for IgG, in batch mode or in-column chromatography at process-level flow rates (Bulletin 5729). Importantly, UNOsphere SUPrA has low nonspecific binding and very fast mass transfer properties, making it a flexible and effective tool for any affinity-based capture application.

Here we demonstrate the use of UNOsphere SUPrA media in polyclonal and monoclonal antibody purification in both batch and column chromatography modes and at process-scale flow rates.

Materials and Methods

Chromatography Media

Bio-Scale™ Mini UNOsphere™ SUPrA and Bio-Scale™ Mini Affi-Prep® 1 ml cartridges are from Bio-Rad Laboratories, Inc. nProtein A Sepharose 4 Fast Flow, Hitrap Protein A HP (1 ml cartridge), and MabSelect media are from GE Healthcare.

Immunoprecipitation

Protein A beads (10 µl) were washed twice with 1 ml buffer (20 mM HEPES, pH 7.5, 50 mM KCl, 50 mM NaF, 1 mM active sodium vanadate, 1 mM sodium molybdate, 2 mM imidazole, 250 mM sucrose, and 1 mM EDTA) before addition of 10 µg anti-GFP antibody. After incubation at 4°C for 2 hr with constant agitation, 0.5 g of plant tissue extract prepared in 1 ml buffer containing 0.5% Triton X-100 and protease inhibitors (1 mM PMSF, 1x cocktail) were added. After incubation at 4°C for 30 min, the beads were washed 4x with 0.5 ml buffer with 0.1 % Triton. Bound proteins were eluted in 50 µl of SDS sample buffer, prewarmed at 95°C, by centrifugation. 25 µl of each eluate along with load were analyzed by SDS-PAGE and western blot against anti-GFP.

Lab-Scale Antibody Purification

Human serum (HMSRM, Bioreclamation Inc., total protein content 68 mg/ml) was diluted 1:5 in phosphate buffered saline (PBS) and filtered through a 0.2 micron membrane. IgG was purified from the serum by Protein A affinity on the Profinia™ protein purification system (Bio-Rad) using the preprogrammed Protein A & G plus desalting method at a flow rate of 1 ml/min (Bulletin 5701 and Berardini et al. 2008).

Protein Analysis

IgG yield was measured for each chromatography run by measuring the absorbance at 280 nm (A_{280}= 1.4 for a 1 mg/ml). Purity of the IgG fraction was measured from Coomassie stained SDS-PAGE Criterion™ 4–20% Tris-HCl gels followed by Coomassie staining using a GS-800™ USB calibrated densitometer and Quantity One® 1-D analysis software (Bio-Rad). The saturation point for each cartridge was estimated by hyperbolic curve fit of the yield data for each cartridge.

Monoclonal Antibody Purification

Cell culture supernatant containing monoclonal antibody was processed through either SUPrA or MabSelect (GE Healthcare) media packed in 1 ml Bio-Scale Mini™ cartridges using a BioLogic DuoFlow™ chromatography system. After 10 column volumes of equilibration (1x PBS) and sample loading, the column was washed with 15 volumes of 1x PBS. Monoclonal antibody was eluted with 10 volumes of 0.1 M glycine, pH 3.0. The flow rate used for all steps was 300 cm/hr (1.23 ml/min).

Results and Discussion

Protein A Mediated Immunoprecipitation of GFP

Anti-GFP antibodies were captured in solution on UNOsphere SUPrA and nProtein A Sepharose 4 Fast Flow media and used to immunoprecipitate GFP from a plant protein extract. Immunoprecipitated GFP was eluted and analyzed by western blot. Our results indicate that UNOsphere SUPrA and nProtein A Sepharose 4 Fast Flow media both effectively captured the GFP antibody as shown by the strong signal for GFP on western blot (Figures 1, 2, and 3).

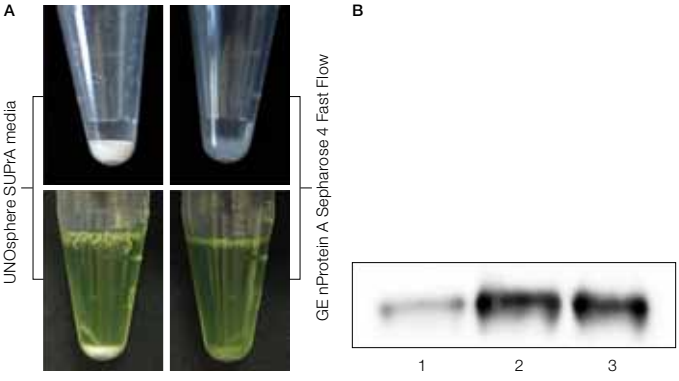


Fig. 1. Capture of GFP by Protein A-GFP antibody. **A**, Pellet of UNOsphere SUPrA and nProtein A Sepharose 4 fast flow media following centrifugation in plain buffer or crude plant extract extract. **B**, Western blot analysis of eluate. The presence of GFP in plant extract eluate was detected using anti-GFP polyclonal antibodies. 1, plant extract; 2, nProtein A Sepharose 4 fast flow eluate; 3, UNOsphere SUPrA media eluate.

However, if we compare the two different Protein A media when mixed either in plain buffer or with plant extracts, the UNOsphere SUPrA media has the advantage of making a tight, clearly visible pellet, making washes easier and minimizng the loss of Protein A media during the washes (Figure 1A).

UNOsphere SUPrA Media Binding Capacity for IgG

Different volumes (5, 10, 15 and 20 ml) of diluted human serum were loaded onto 1 ml UNOsphere SUPrA, HiTrap Protein A HP, and Affi-Prep Protein A cartridges. Purified IgG fractions were analyzed on SDS-PAGE for quantitation and purity assessment (Figure 2). The three different media tested effectively captured the IgG antibody from the human serum (Figures 2A and B). However, UNOsphere SUPrA demonstrated a higher dynamic binding capacity (25 to 30 mg/ml) than either HiTrap Protein A HP or Affi-Prep Protein A with no loss of IgG purity (Figure 2C).

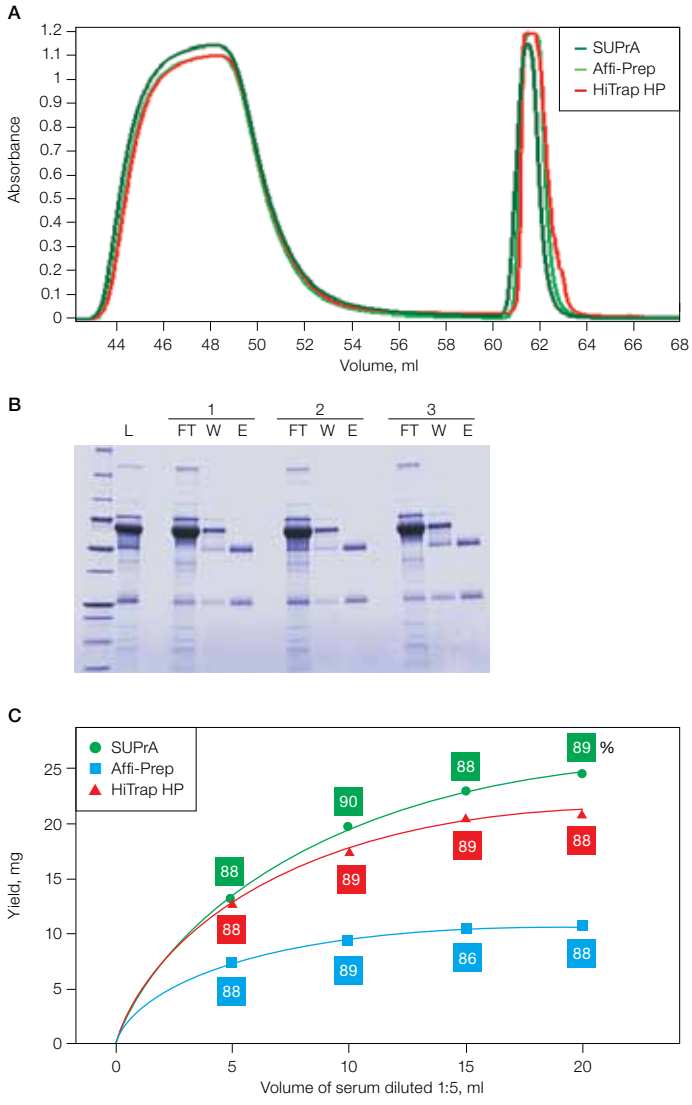


Fig. 2. Lab-scale purification of human IgG from serum using the Profina protein purification system. A, Chromatogram showing overlayed purification profiles of IgG using three different Protein A media. **B**, SDS-PAGE of purified IgG. 1, SUPrA; 2, Affi-Prep; 3, HiTrap HP; L, Load; FT, flow through; W, wash; E, eluate. **C**, Plots of dynamic yield of IgG purified using different Protein A media cartridges. % Purity of each fraction is shown in adjacent boxes.

Purification of Monoclonal Antibody with UNOsphere SUPrA Media

UNOsphere SUPrA media is also very effective for the purification of monolconal antibodies. We compared the performance of UNOsphere SUPrA media with that of MabSelect Protein A media. The results indicate that both media yield similar amounts and purity of mAb, with low levels of host cell proteins and DNA contamination (Figure 3 and Table 1). Previously we have demonstrated that UNOsphere SUPrA media is a powerful tool for process-scale antibody purification when combined with UNOsphere Q and CHT™ ceramic hydroxyapatite media (He et al. 2008).

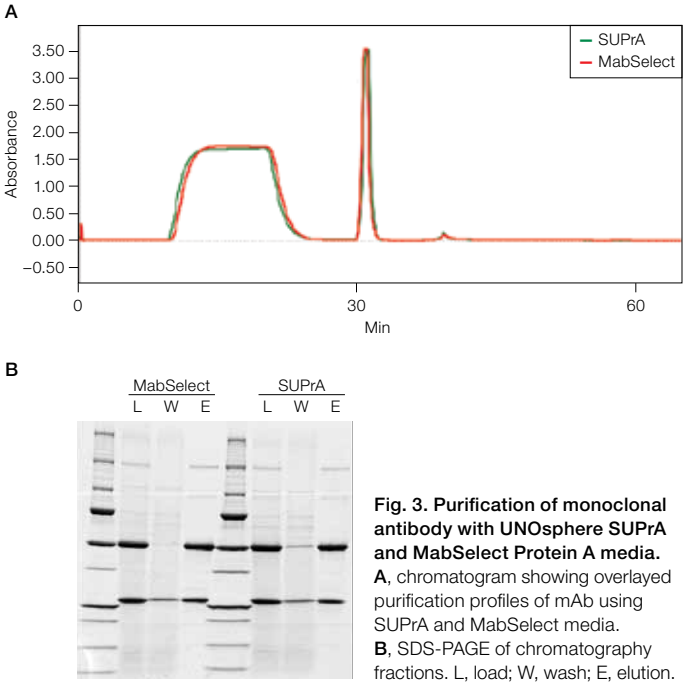


Fig. 3. Purification of monoclonal antibody with UNOsphere SUPrA and MabSelect Protein A media. **A**, chromatogram showing overlayed purification profiles of mAb using SUPrA and MabSelect media. **B**, SDS-PAGE of chromatography fractions. L, load; W, wash; E, elution.

Table 1. Quantitation of remaining HCP* and DNA from single step monoclonal antibody purification by UNOsphere SUPrA and MabSelect

Media	HCP (ng/mg)	DNA (ng/mg)	rPA (ppm)
MabSelect	39.2	26.0	6.4
SUPrA	33.2	21.6	20.5

* HCP, host cell protein.

In summary, UNOsphere SUPrA media gives similar dynamic binding capacity and purity to leading competitor products over a range of different laboratory applications. It is an excellent choice for use in protein immunoprecipitation assays and for both laboratory and process-scale purification of polyclonal and monoclonal IgG antibodies.

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EFFICIENT ELECTROPHORESIS

By Shawn Miller

Aiding Advances in Cardiomyopathy Research

The electrophoretic workflow has undergone few changes since it was solidified as a fundamental tool in life science research in the 1970s. Today, just like then, researchers need to prepare samples, load gels, then perform separation and further downstream analysis, including western blotting. But because the pace of research has accelerated over the past several decades, it has become essential to develop ways to shorten these steps without sacrificing the quality of results. In recent years, Bio-Rad has met this need with the introduction of the Mini-PROTEAN® TGX™ gels (long shelf life gels in Laemmli formulation), the Gel Doc EZ imaging system (one-button operation), and now, the Trans-Blot® Turbo™ transfer system.

Recently, researchers in the Aldrin Gomes laboratory at the College of Biological Sciences at UC Davis agreed to assess the potential impact of the Trans-Blot Turbo system on their efforts to further understand the role of proteasomes and troponins in cardiac and skeletal muscle tissues, as well as in protein degradation and cardiomyopathies.

Foundation in Electrophoretic Efficiency

A native of Trinidad and Tobago, Dr Aldrin Gomes decided to specialize in the biochemical differences between normal and diseased hearts in his graduate studies at the University of the West Indies. A significant portion of his research utilized protein separation and purification techniques, making SDS-PAGE electrophoresis an essential tool in most of his experiments. But in a country where resources are scarce, unrelenting heat is destructive to sensitive biological materials, and slow delivery times are common for product orders, Gomes and his colleagues began to analyze the electrophoretic workflow to determine how to make the process as efficient as possible. "Because we performed a lot of electrophoresis, we published some articles whereby we looked at how we could standardize things to improve resolution in our results," says Gomes. "We heavily researched aliquoting methods, sample buffers (methods for making them and determining actual shelf lives), whether or not buffers can be reused — even minor factors such as gel pouring techniques and plate thickness."

Since Gomes "grew up," scientifically speaking, in an environment where experiments must be planned far in advance and resources cannot be wasted, he cultivated the habit of designing experiments and procedures that made the best possible use of tools and time while ensuring optimal results. This followed him through his graduate work and his subsequent career, first as a research associate, then in his current role as assistant professor in the Neurobiology, Physiology and Behavior department in the College of Biological Sciences, and Physiology and Membrane Biology department in the School of Medicine at UC Davis.

Making Strides in Cardiac Disease Research

Drawn to UC Davis by the convergence of scientific disciplines and the school's reputation, it is here that Gomes has been able to make significant strides in his field of research. "You hear about young people who exercise and look fine on the outside, but die suddenly in the middle of strenuous exercise from heart attacks," says Gomes, explaining the driving force behind his research into troponin, a complex of three proteins involved in cardiac muscle contraction. "They look just like us: happy, healthy, no signs of any problem, but tomorrow they are gone." Gomes explains that many of these people have cardiomyopathies that manifest without causing the heart to enlarge, so the condition is not detectable by x-ray or ultrasound; instead, these types of cardiomyopathies are only visually evident in a biopsy. So developing a way to detect the condition prior to a sudden death episode is critical. That's where Gomes's research comes into play.

The genetics of most forms of cardiomyopathies are known; a significant percentage of people who are victim of the sudden cardiac death described above have mutations in muscle proteins. Researchers in Gomes's lab spend a significant amount of time studying the molecular mechanisms of these mutations. "We have transgenic mice that we are studying, and we know a lot about what is happening," says Gomes. "We know that the troponin mutation changes calcium sensitivity, so the heart cannot respond as necessary. We know that a lot of ion channels affect overall calcium, changing proteolysis to some degree. We are trying to fit all the pieces together as well as build a model to predict susceptibility to cardiomyopathy-related sudden death from too much stress on the heart." An important goal of this research is to develop

For more information, request **Bulletin 6039**.

Making Electrophoresis and Blotting More Efficient One Product at a Time

Trans-Blot Turbo System Gives the Best Transfer Efficiency

Trans-Blot Turbo System	Traditional Semi-Dry Transfer	Tank Transfer
		
7 minutes	**30** minutes	**60** minutes

The Trans-Blot Turbo system delivers better transfer results than the gold standards — transfer efficiencies of the Trans-Blot Turbo system, Trans-Blot SD semi-dry transfer cell, and Mini Trans-Blot transfer cell. Precision Plus Protein™ WesternC™ standard was loaded on a 4–20% Mini-PROTEAN TGX precast gel (5 µl, lanes 1 and 10), along with a 2-fold dilution of 160–1.25 ng of human transferrin (5 µl, lanes 2–8). The Mini-PROTEAN TGX gel was run at 200 V for 30 minutes and transferred with three different methods. **A**, Trans-Blot Turbo system transfer, the proteins from the gel were transferred to a PVDF membrane at 2.5 A, 25 V for 7 minutes; **B**, semi-dry transfer, the proteins from the gel were transferred to a PVDF membrane at 25 V for 30 minutes; and **C**, tank transfer, the proteins from the gel were transferred to a PVDF membrane at 100 V for 30 minutes. All blots were blocked, probed, detected, and imaged on a VersaDoc™ 4000 MP imaging system; images were adjusted for the best signal to background.

New Innovations from Bio-Rad

- **TGX gels** — long shelf life midi format gels for PAGE maintain cooler temperatures at high voltages, allowing run times as short as 20 minutes. Designed to provide Laemmli-like separation patterns using the standard Tris/glycine/SDS running buffer system, these gels are available in both traditional and stain-free formats

- **Trans-Blot Turbo transfer system** — combines traditional semi-dry blotting techniques with filter paper and buffer, allowing fast transfer of proteins with minimal preparation time. By providing the entire system in a ready-to-use format, it allows researchers to obtain results faster and easier, with reproducibility that is difficult to achieve by traditional tank and semi-dry blotting methods

- **Gel Doc EZ imaging system** — compact and automated system for obtaining publication-quality images and analyzed results in minutes with the push of a button. The four application-specific trays allow UV, Coomassie, copper, silver, and zinc stain, nucleic acid, and stain-free imaging.

screening methods that can be utilized to determine the presence of mutations and predict the chances for developing severe cardiomyopathies.

The other major research focus in the Gomes lab is the proteasome, the main proteolytic system inside the body that is responsible for degradation of ~80% of all proteins. Specifically, researchers are interested in studying the role of the proteasome in normal and diseased hearts. “In practically every disease that is studied today,” says Gomes, “the proteasome is affected. In some diseases the proteasome is directly involved (Parkinson’s, Alzheimer’s); in others it may be secondary, but it’s involved in everything.” The primary objective is to identify phosphorylation sites that activate and inactivate the proteasome; a secondary effort is searching common compounds that affect its activity. “We have some interesting findings in this regard,” says Gomes. “For example, we have found that several commonly used drugs affect the activity of the proteasome. Proteasome inhibitors have been shown to cure cancer (multiple myeloma), but they can cause cardiac toxicity. So we’re studying these things on multiple fronts.” It is this leg of his research that relies the heaviest on electrophoresis.

Impact of Latest Electrophoresis Tools

In the Gomes laboratory, the need for quality and improved efficiency drives all purchasing decisions. Especially when it comes to electrophoresis, the goal is to perform this fundamental technique with minimal resources — primarily in terms of time and labor — without impacting reliability or reproducibility. Gomes says that in addition to efficiency and quality, his product acquisition decisions are determined by convenience, referring back to his graduate student days when optimizing electrophoresis conditions required flexibility in consumable usage. It is this need for flexibility regarding the use of buffer systems that caused the Gomes lab to switch to the use of Bio-Rad’s Mini-PROTEAN TGX gels. “In terms of our electrophoresis workflow,” explains Gomes, “these gels offer time savings, high quality, and use traditional instead of specialized buffer systems.”

Since western blotting technology was developed more than 30 years ago, there has been little innovation in this step of the electrophoresis workflow. Bio-Rad has developed the new Trans-Blot Turbo transfer system to meet a need for greater flexibility. Both high– and low–molecular weight proteins can be transferred using both rapid turbo and traditional semi-dry techniques. Moreover, results in this high-throughput system can be obtained in as little as 3 minutes.

Prior to testing the Trans-Blot Turbo system, researchers in the Gomes lab had primarily been relying on tank blotting, a process that can take as little as an hour, but sometimes requires overnight transfers. In addition, their tank blotting apparatus can only perform two transfers at a time and has high power requirements. With only three power supplies that meet these requirements (which are shared among other devices), the blotting step has been a major bottleneck in their workflow. To conduct his test of the Trans-Blot Turbo system, Gomes ran a protein gel, transferred the proteins to a membrane, then performed Ponceau staining.

The Trans-Blot Turbo system has the capacity to run four mini or two midi gels at one time (see page 2 for complete system capabilties). Results obtained with the Trans-Blot Turbo system are comparable to those obtained with current methodologies, but it takes only 7 minutes. In addition, the Trans-Blot Turbo system has its own built-in power supply. “How I see the Trans-Blot Turbo system helping labs like mine in the blotting step is that it eliminates dead time,” says Gomes. “Now we can do everything in 45 minutes, from starting a gel to transblotting. So instead of 10 electrophoresis experiments over the course of a week, we can now do up to 12 in one day — the rest of the time we don’t have to make routine experiments an important part of our research. Instead, we can focus on important findings.”

Conclusions

Research throughput requirements as well as a history of analyzing the electrophoresis and blotting workflow for improvements made the Gomes lab an ideal location for testing the Trans-Blot Turbo system. This latest blotting innovation by Bio-Rad offers flexibility, researchers can choose either a rapid protocol or perform a standard semi-dry transfer, as well speed and high-throughput. As Gomes has demonstrated, these features do not come at a sacrifice to the quality of the transfer.

Dr Aldrin Gomes testing the Trans-Blot Turbo system. Transfer occurred in 7 minutes compared to the 1 hour that is typical with tank blotting; the quality of results was comparable in both methods.

Research Applications for the EpiQ™ Chromatin Analysis Kit

The EpiQ chromatin analysis kit is a novel epigenetics research tool for the quantitative assessment of chromatin structure in cultured cells. By combining *in situ* chromatin digestion, genomic DNA purification, and real-time PCR, the chromatin state for several gene promoters can be studied simultaneously. The EpiQ kit helps quantify the impact of epigenetic events such as DNA methylation and histone modification on gene expression regulation through chromatin state changes. Genes that are actively transcribed are associated with "open" or "accessible" chromatin regions, while genes that are transcriptionally silent are often in "closed" or "inaccessible" chromatin regions (Figure 1).

The EpiQ chromatin analysis kit discriminates between open and closed regions by employing a nuclease accessibility assay and real-time PCR to quantify the level of accessibility. Rapid results can be obtained in approximately 6 hours and strongly correlate with gene expression levels (Figure 2). The EpiQ kit complements existing epigenetic assays (DNA methylation and histone analysis using chromatin immunoprecipitation), and researchers worldwide are using the kit to obtain novel insights into mechanisms of gene regulation.

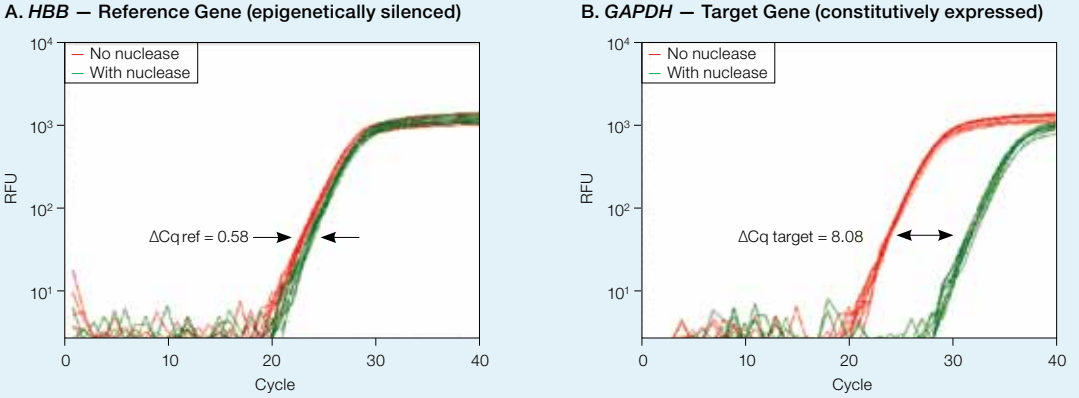

Fig. 1. The EpiQ chromatin analysis kit utilizes nuclease accessibility to discriminate open vs. closed chromatin regions. Amplification of proximal promoter regions for the epigenetically silenced *HBB* (reference) gene or the constitutively expressed *GAPDH* (target) gene was carried out in HeLa cells using the EpiQ kit and EpiQ™ chromatin SYBR® Green supermix on the CFX96™ real-time PCR detection system. **A**, closed chromatin regions resulted in minimal quantification cycle (Cq) delays (ΔCq = 0.58) following nuclease treatment; **B**, open chromatin regions led to significant Cq delays (ΔCq = 8.08) after nuclease treatment. RFU, relative fluorescent units.

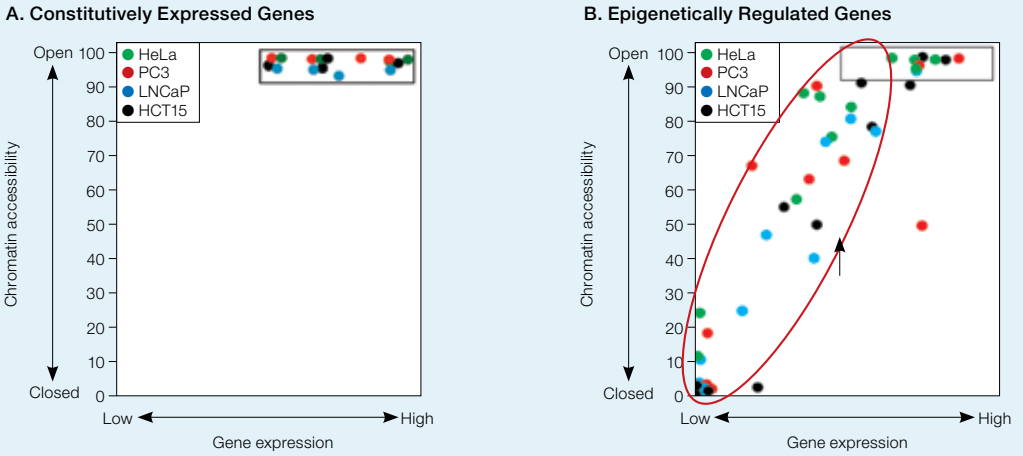

Fig. 2. Chromatin structure data from the EpiQ chromatin analysis kit strongly correlate with gene expression levels for several genes in various cell lines. A, the chromatin state of the proximal promoter regions for four constitutively expressed genes (*GAPDH*, *ACTB*, *TBP*, and *B2M*) was assessed in four different cell lines (HeLa, PC3, LNCaP, and HCT15). Each gene promoter was determined to be in an open chromatin structure. **B**, eleven epigenetically regulated gene promoters (ABCB1, CDH1, CDH13, DAPK1, DKK1, GSTP1, PTGS2, TP73, WT1, p14ARF, and p16INK4A) were evaluated for chromatin structure in the same four cell lines. Chromatin state varied across the panel of genes and strongly correlated with the gene expression levels. Promoter regions in an open state were associated with medium to high levels of gene expression (shown in the black box, upper right), whereas those in a closed state were linked to low expression (lower left).

Ian Marc Bonapace, PhD
Department of Structural and Functional Biology
University of Insubria
Varese, Italy

Making Connections in Cancer Cell Proliferation

Together with a group of Japanese researchers, Dr Ian Marc Bonapace discovered the UHRF1 protein in the late 1990s. At that time, Bonapace was searching for proteins involved in the control of cellular differentiation and proliferation. Since its discovery, UHRF1 has been shown to play an important role in gene regulation; specifically, in controlling the epigenetic machinery that reinstalls correct modifications on DNA and chromatin during cell proliferation.

The main objective of Bonapace's studies is determining ways UHRF1 can ultimately affect cancer treatment. "We believe at minimum, that this protein can be very useful as a marker for cancer aggressiveness," explains Bonapace. "But what we hope to discover in the future, is that hampering the activity of UHRF1 could be a way to stop tumor growth and metastasis in the body."

Some of the primary techniques used in this research include chromatin immunoprecipitation, protein extraction and purification, and coimmunoprecipitation and gel filtration to identify the complexes in which this protein is involved. Early in 2010, Bonapace obtained the EpiQ kit. To test its efficacy and applicability to his overall research efforts, he conducted an experiment to compare the expression of three different oncosupressors in two different lines of prostate cancer (LNCAP, a line that is not aggressive, and PC3, a line that comes from a very aggressive tumor). "The results of these tests showed what we expected to see based on previous research," says Bonapace. "The oncosuppressors were not downregulated in LNCAP cells, but completely downregulated (so chromatin was in a closed state) in PC3 cells."

Bonapace explains that prior to obtaining the EpiQ kit, determining if genes are being expressed or not, required days of work using the more resource-intensive chromatin immunoprecipitation method. "With this kit," he explains, "you can determine immediately if genes are repressed or not. Then you know whether or not to go through the more time-consuming and money-consuming experiments to study why." Bonapace describes the potential impact of EpiQ kit technology on his research as providing significant time savings and efficient use of resources as he continues to test different kinds of cells and tumors to see if the same genes of interest are or are not controlled by the presence or absence of the UHRF1 protein. "The main application of this kit is not so much to aid in the discovery of something," he says, "but more to provide a shortcut to understanding if a gene is expressed or not. Once you've understood that, you can go on to design studies to define better and in more detail why."

Siddharth Dey
Graduate Student
David Schaffer Laboratory
University of California, Berkeley

Determining the Mechanisms of HIV Latency

When Siddharth Dey first came to UC Berkeley for graduate school, he came across a paper published in *Cell* by researchers in the Schaffer lab that proposed a novel model of HIV latency. The paper demonstrated that the stochastic fluctuations in a virally encoded protein that binds to a viral RNA hairpin to activate transcription, thus forming a positive feedback loop of transcription, could influence the viral latency decision. Initially, the number of these viral molecules are very low within the cell. The new model proposed that there are two options for whether a viral protein can bind to the RNA hairpin and drive transcription, giving rise to proliferation of the viral protein. It either starts a positive feedback loop of transcription and activates the virus, or the initial low numbers of the viral protein can fail to activate the positive feedback loop, rendering the virus latent. The latent viruses are not susceptible to antiretroviral drugs, but the positive feedback loop can activate at a later time, making it impossible to completely eradicate the virus from infected patients. It was this new model of HIV latency that piqued Dey's interest in studying

the evolution of these viral proteins and made him decide to devote his graduate studies to work in the Schaffer lab.

Since joining the lab, Dey has been working on the evolution of HIV. "HIV is divided into various subtypes," says Dey, "and it has a very high error rate, so basically you have this huge diversity. One of my goals is to understand how this diversity affects function, and how the virus still manages to operate so efficiently in spite of the variability: are there some underlying structural factors that are important for protein function?"

> "The EpiQ kit made it possible for me to evaluate the chromatin structure around the integrated virus, and whether the viral protein affects it."

Dey is also interested in applying research that has helped characterize strains of HIV that infect people in the U.S. or Western Europe to strains that infect other world populations that are far less studied. "I started looking into how these different processes that got me first attracted to the lab can vary when you have different sequences," he explains. "Essentially, how does sequence diversity within this protein affect various pathways or gene expression of the virus?"

In recent experiments, Dey studied a viral protein that has been shown to exhibit significant posttranslational modification and interact with a variety of cellular proteins. Several conserved sites within the protein have been identified as critical for the protein to function and if these are mutated, the virus becomes nonfunctional. "I was interested in looking one step ahead and identifying potentially non-conserved sites that may be functionally important. You can have situations where not just one site is conserved, but two sites simultaneously are conserved, so sites can co-evolve. In other words, amino acids at certain sites are correlated with amino acids at other sites."

Part of this work required applying statistical methods on sequence alignments of the protein to identify sites that are co-evolving. Once potential sites were identified computationally, Dey was able to test these sites experimentally. "These sites definitely looked like they were co-evolving, which told us that if you just look at a sequence alignment, they may not look functionally important on a site-to-site basis, but there's definitely correlation between the sites," explains Dey. "Once we identified these co-evolving sites and verified them experimentally, the next step forward was to ask: how are they important either structurally or functionally?" Further experimentation demonstrated functional attributes that these co-evolving sites satisfy. If the co-evolving pairs are disrupted, the viral protein loses its function.

The EpiQ kit played a central role in these functional experiments. The viral protein has been shown to interact with many cellular proteins, including SWI/SNF, a chromatin remodeling complex. "So I attempted to determine how mutations within the viral protein affect the way in which it interacts with the chromatin remodeling factor," explains Dey. "The EpiQ kit made it possible for me to evaluate the chromatin structure around the integrated virus, and whether the viral protein affects it." In the past, Dey used chromatin immunoprecipitation to address similar questions. For the purposes of this and his ongoing research, the EpiQ kit does not replace this technology, rather it complements it, giving insight into the circumstances under which further analysis using chromatin immunoprecipitation is warranted.

Dey is hopeful that the questions being asked in the Schaffer lab related to HIV will eventually lead to insights into why HIV might be present in a person who does not exhibit active signs of disease. "Ultimately," he explains, "we'd like to be able to completely understand what is happening at the molecular level in terms of HIV latency, so mechanisms can be developed to activate the virus completely, then apply an antiretroviral drug to kill it off."

Nuvia™ S Media — A High-Capacity Cation Exchanger for Biomolecule Purification and Downstream Processing

Introduction

Nuvia S media is a recently introduced, high-capacity, strong cation exchanger (CEX) developed to operate over a range of experimental conditions, allowing scientists to scale up from laboratory- to process-scale more quickly and easily. Nuvia S is based on a large pore hydrophilic matrix produced by polymerization of water-soluble, hydrophilic acylamido and vinylic monomers. Such an approach to design offers Nuvia S media excellent dynamic binding performance (100–170 mg/ml) for target biomolecules and low backpressure at the high flow-rates (150–600 cm/hr) used in biomolecule purification. These properties, therefore, give Nuvia S media the robustness and productivity required for initial capture of monoclonal antibody (mAb) from cell culture harvest as well as the impurity clearance capability needed for the purification of mAbs and other protein therapeutics.

Tolerance to Variations in Load Composition

mAbs are generally produced by mammalian expression cultures at physiological pH. A typical mAb harvest also contains a substantial amount of sodium chloride, which requires pH adjustment and inline dilution before loading onto a cation exchange column. The tolerance of load variation by Nuvia S media has been studied in the conductivity range of 4–12 mS/cm at pH 5.0, which corresponds to 15–105 mM of sodium chloride in solution. The 5% and 10% breakthrough binding capacities (BBC) of prepurified monoclonal antibody mAb1 varied only slightly — from 140 to 180 mg/ml at 300 cm/hr (Figure 1). Since the pI value of an antibody as well as the pH and conductivity of the product pool determine the strength of its interaction with the ligands on CEX media, users are advised to examine the dynamic binding capacity of a mAb in expression harvest at multiple pH-conductivity combinations using design of experiments (DoE) during process development and optimization.

Removal of mAb1 Aggregates During Intermediate Polishing

mAb1 tends to aggregate during the acidic elution step of Protein A affinity capture chromatography. A salt-step elution method was developed to recover monomeric mAb1 while retaining aggregates on the Nuvia S column until stripping with 1 N NaOH (Figure 2). The overall yield of monomeric mAb1 in such chromatographic preparation was 93%, and the aggregate contamination level was effectively reduced from 13.8% in the load to <1% in the eluate (Figure 2, inset).

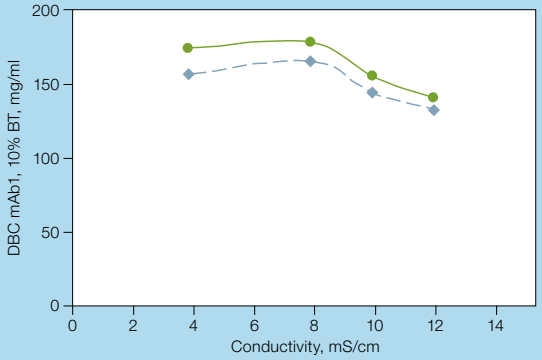

Fig. 1. Dynamic binding capacity of monoclonal antibody mAb1 by Nuvia S media. Binding of a monoclonal antibody (mAb) by Nuvia S media; column size 1.1 x 10 cm; sample mAb1 (4.5–5.0 mg/ml) was loaded onto the column in 20 mM sodium acetate, pH 5.0 + sodium chloride (adjusted for conductivity) until 5% (◆) and 10% (●) breakthrough capacity were observed.

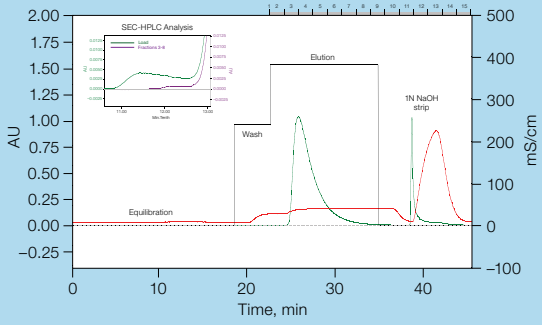

Fig. 2. Removal of monoclonal antibody mAb1 aggregates by Nuvia S media. Equilibration buffer, 20 mM sodium acetate and 20 mM sodium chloride, pH 4.7; wash buffer, 20 mM sodium acetate and 160 mM sodium chloride, pH 4.7; and elution buffer, 20 mM sodium acetate and 245 mM sodium chloride, pH 4.9. Linear flow rate, 300 cm/hr. (—), OD_{280}; (—), conductivity; (—), salt steps. Inset, (—), load; (—), fractions 3–8.

Conclusion

Increasing productivity and reducing costs continue to be the driving forces in process development. Recent advances in upstream processes have dramatically improved the efficiency of cell cultures. However, prolonged growth or cultivation and high concentration of mAbs at harvest may also lead to product degradation and/or aggregation. Clearing these unwanted by-products remains one of the main challenges in downstream processing of therapeutic mAbs. Our data demonstrate that Nuvia S media is suitable for both capturing and polishing steps in the manufacturing of mAbs over a wide range of operating conditions. Its high capacity and superb purification performance can significantly improve productivity by offering the robustness, capacity, and speed for processing large load volumes with smaller columns and less buffer consumption.

Gel Doc™ EZ Imaging System – A Tale of Speed and Simplicity in Two Cities

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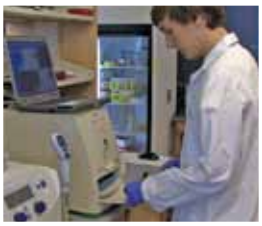

From top to bottom: Faneuil Hall, Boston; Nikolai Eroshenko using the Gel Doc EZ system; Harvard Medical School main campus; Sriram Kosuri performing a PCR experiment.

Boston, Massachusetts

Cradle of Discovery

Known as the cradle of democracy for its role in shaping the events of the American Revolution, in more recent history Boston has become an important center for the genesis of revolutionary concepts in scientific research. At the forefront of these discovery efforts is the Wyss Institute for Biologically Inspired Engineering at Harvard University. Among the institute's core faculty members is George Church, PhD, professor of genetics at Harvard Medical School. Church is probably most known for spearheading much of the early stages of the Human Genome Project. But parallel to this work in biological discovery, Church has devoted his career to developing technologies to engineer and synthesize genes in much faster, easier ways. It was to this cutting-edge environment, in which life science research is combined with engineering, that Sriram Kosuri was drawn as a research associate.

"The Wyss Institue was just starting when I joined," Kosuri says, "and because George's lab is based on developing new technologies and exploring high-risk technologies for biological engineering, it seemed like the ideal place in which to conduct research."

Some projects include the Personal Genome Project, an effort to sequence and publish individual genomes; the production and differentiation of human stem cell lines for various types of therapeutics; discovery of novel pathways for better biofuels; and the arbitrary engineering of a genome. "The lab is very diverse in its applications," says Kosuri, "but the unifying theme is technology development to find solutions to biologically inspired problems that have been vexing."

One of the Church lab's primary goals is to improve technology so that basic techniques can be performed affordably and automatically — allowing researchers to focus on discovering answers to new questions instead of being inhibited by the resource requirements of basic research. Kosuri refers to the original genome project, which took 10 to 15 years to complete at a cost of several billion dollars, and explains that Church has been exploring methods in which a similar effort can be conducted in months and at a cost of approximately $1,500.

Because primary research in the Church lab is centered on the manipulation and detection of DNA, nucleic acid–based applications (PCR, agarose gel electrophoresis and imaging, sequencing, and DNA synthesis) underlie a majority of their experimental efforts.

Kosuri describes time as the main obstacle to achieving their objectives. "There are only so many people and so many hands," he says, "so everything we do is geared toward reducing the time we spend doing basic science." Because of this critical need, Kosuri agreed to beta test the automated Gel Doc EZ imaging system, developed for obtaining images from a variety of applications with just the push of a button. Prior to the Gel Doc EZ system, the lab had been using a chemiluminescence imager. "A very nice system if you want to take a 20-minute exposure and really look at low noise," says Kosuri. "But the fact is, most of what we do is standard agarose gel electrophoresis." The more complex chemiluminescence system required researchers to focus the camera, set the stage, and use the right filters. "When we got the opportunity to try the EZ imaging system, what ended up happening was everyone switched over to it," says Kosuri. "There's no fussing with zoom and focus; it automatically does most of that, and sets the exposure, and the images are great, so you can use them in publications. It's taken over most of the day-to-day responsibilities of the chemiluminescence machine and opened up that machine for the images it's supposed to take."

Since each image now takes seconds versus 5 to 10 minutes with the chemiluminescence system, Kosuri estimates that the Gel Doc EZ system easily saves hours in a day. Plus, the system sits on the bench next to where they run their gels. "So we can quickly see if our gel has run long enough, which has actually changed the way we use the gel imager," says Kosuri. "We don't just use it for our final images."

Kosuri says that the Gel Doc EZ system has become an important tool for what research in the Church lab requires everyday — taking a lot of agarose gel images. "It's become as essential as a PCR machine, so it'll play a part in everything we do — mostly because it's fast and works very well."

Dorota Krawczyk, PhD
Assistant Project Scientist
Geoff Rosenfeld Laboratory
Howard Hughes
Medical Institute
University of California,
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San Diego, California

Biotech Beach

It was during her work as a graduate student in Geneva, Switzerland that Dr Dorota Krawczyk was first exposed to the research of Dr Geoff Rosenfeld, principal investigator with the Howard Hughes Medical Institute at the University of California, San Diego's School of Medicine. While studying retinol development, Krawczyk became fascinated with applying molecular biology to the understanding of basic mechanisms and how things develop (such as cellular determination and differentation, and the signalling pathways or transcription factors involved at the molecular level in these processes). In the course of her early research, she came across several articles dealing with very difficult questions in development at the molecular level. "I realized that most of the articles on this topic were coming from one lab," says Krawczyk, "so it became obvious to me that if I wanted to continue in this field, I needed to go to the person that is doing work on the highest levels, applies the newest technologies, and is not afraid to tackle very difficult biological questions."

Krawczyk joined the Rosenfeld lab to study pituitary development and describes the environment as one of few in which the research of molecular biology is applied to the study of the molecular mechanisms that drive development. The lab is perhaps best known for its work on nuclear receptors and how they influence gene expression and relate to disease research, mostly breast and prostate cancer. A recent project is exploring how different factors can influence the reorganization of the chromatin in the nucleus. "We noticed that this can happen in the development of cancer," explains Krawczyk, "but it can also happen in the development of healthy tissue, so what we're interested in understanding is how/if nuclear organization influences the development of the cell or the cancer."

Some of the techniques commonly used by researchers in these studies include protein isolation, expression, and detection, as well as RNA expression and detection of the various factors of interest, most often by performing qPCR and reverse-transcription qPCR. More complex experimental methods include isolation of protein complexes, detection of interactions between the proteins, and chromatin immunoprecipitation, very often coupled with high-throughput sequencing.

Because research here involves both protein and nucleic acid studies, the Rosenfeld lab requires basic tools — including an imaging system — that offer versatility as well as resource-efficient functionality. Before acquiring the Gel Doc EZ system, researchers in Krawczyk's laboratory had access to an older imaging system located up one flight of stairs. "The whole process took time," says Krawczyk, "not only walking there, but taking the picture, adjusting the conditions, then coming back to the lab and realizing, 'Oh no, I would prefer to have it differently' — then going upstairs again." Therefore, the opportunity to work with the Gel Doc EZ system was most welcome. "What was surprising for me," says Krawczyk, "is that without a big introduction to the system, we were able to just start using it right away."

Among the features that researchers in the Rosenfeld lab have appreciated with the Gel Doc EZ system is its speed. It takes 20 to 40 seconds to obtain an image and ~one minute to perform basic image analysis. Versatility is another benefit. The Gel Doc EZ system's application trays enable researchers to image both nucleic acid as well as protein gels with different types of stains. "With the other system I was reluctant to take a picture unless I knew it would really be the final one," says Krawczyk. "Now I can interrupt the migration at any point to take a picture and see if it's ok or not."

The Rosenfeld lab plans to continue its understanding of developmental mechanisms. "We are working on basic science, asking very basic questions," says Krawczyk. "This is not application-driven work. However, we cannot forget that in the end, our answers can potentially be used in therapies that solve problems for patients dealing with disease."

From top to bottom: Torrey Pines State Natural Reserve, San Diego, California; Gel Doc EZ with tray; entrance to the UCSD campus; Dorota Krawczyk imaging a gel on the Gel Doc EZ imager.

The "Opportunarray" of a Lifetime — ProteOn™ XPR36 Protein Interaction System 2010 Giveaway Winner Announced

Simon Cocklin, PhD
Research Professor of Biochemistry and Molecular Biology
Drexel University, College of Medicine

Surface plasmon resonance (SPR) studies have helped to elucidate the roles that intracellular concentration, ionic environment, cofactors, and protein conformation play in maintaining complex biological processes. It's 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 integrates state-of-the-art microfluidics with a novel optical design to create a unique 6 x 6 interaction array (Figure 1) for SPR experiments.

On May 15, 2010, Bio-Rad launched a program to stimulate label-free protein interaction research in labs around the world, offering researchers the chance to win a free ProteOn XPR36 protein interaction array system. Contestants submitted short research proposals involving SPR technology that were evaluated by an external panel of experts, including current ProteOn system users and editors for *Genetic Engineering News*. Bio-Rad is pleased to announce the grand prize winner as Simon Cocklin, PhD, research professor for the department of biochemistry and molecular biology at Drexel University College of Medicine. The focus of Cocklin's research is macromolecular interaction analysis, structure-function relationships, and ligand-recptor interactions as they relate to HIV replication.

"I saw the application online and figured, why not fill it out," says Cocklin, "but I never in a million years expected to win." Currently, the institution has one SPR instrument that is shared between Cocklin and another laboratory. "Our current SPR instrument is upstairs from my laboratory and in use 24 hours a day, 7 days a week," says Cocklin. He plans to immediately dedicate the ProteOn system to developing and iteratively improving a series of novel small-molecule inhibitors of HIV-1 replication targeting (1) the HIV-1 Env protein — a protein complex responsible for the entry of the virus into permissive cells and (2) the HIV-1 matrix (MA) protein — a pleiotropic regulator of many essential functions within the viral life cycle.

"One of the main advantages of the ProteOn system is that it gives you the ability to obtain a full kinetic dataset in just one injection," says Cocklin. "This obviates the need to try to scout the regeneration conditions, which is often the key bottleneck in SPR studies."

Jill Raymond, marketing manager for Bio-Rad, explains that Cocklin's application was selected because of the potential impact of the questions he is trying to resolve in the field of HIV research. "To my knowledge, the ProteOn XPR36 Giveaway Program is the first industry competition to award such a high-value prize solely on the basis of scientific merit."

Cocklin notes that in addition to using the instrument in his own lab to further his research efforts, he plans to make it available to other researchers at the College of Medicine.

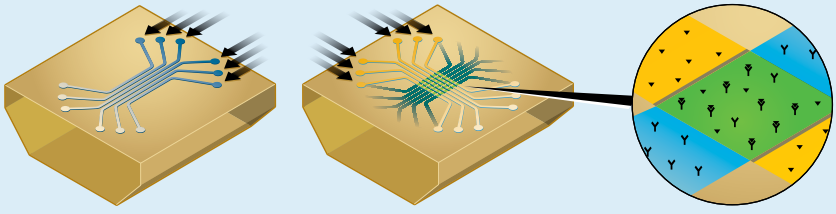


Fig.1. Up to six ligands can be injected through channels in the vertical direction and up to six analytes can be injected in the horizontal direction, producing up to 36 data points in a single experiment. This process also creates unique horizontal interspots that have not been exposed to the ligand 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.

Using the Precision Plus Protein™ WesternC™ Standard as a QC Tool at Key Steps in the Electrophoresis and Blotting Workflow

As a fundamental tool in protein research, it is critical that all steps of the electrophoresis workflow (from running gels to visualizing blots) are performed successfully to ensure accurate, reliable, and reproducible results. Protein standards are often used to monitor electrophoretic separation and transfer efficiency but do not always enable ladder visualization after blot development. Bio-Rad's Precision Plus Protein WesternC standard enables efficient monitoring of all key electrophoresis and blotting steps, allowing quality control checkpoints throughout the workflow. Precision Plus Protein WesternC standards enable you to:

- Visualize and monitor electrophoretic separation to ensure normal progression
- Assess transfer efficiency for western blotting
- Visualize the standard and estimate MW of protein samples after blot chemiluminescence development (with use of Precision Protein™ StrepTactin–HRP or –AP conjugates; Figure 1)
- Visualize standards if using fluorescence detection

Use the following tips for optimizing Precision Plus Protein WesternC standards in chemiluminescence blot experiments.

Tips for Troubleshooting Chemiluminescence Blots

Problem: Bands are too dark upon chemiluminescence development

- StrepTactin dilution was too low for the sensitivity of the chemiluminescence kit used
  - Use a higher dilution of StrepTactin or use a more sensitive chemiluminescence kit*
- The blot was incubated too long with the chemiluminescence substrate
  - Use the recommended incubation time of the substrate kit
- Exposure times were too long

Problem: Bands are too faint upon chemiluminescence development

- StrepTactin dilution was too high for the sensitivity of the chemiluminescence kit used
  - Lower the dilution of StrepTactin or use a more sensitive chemiluminescence kit*
- The blot was not well incubated with the chemiluminescence substrate
  - Make sure that the surface of the blot is well covered with the substrate solution; also, use the incubation time recommended in the substrate kit
- Precision Plus Protein WesternC standard was either over-transferred or under-transferred due to the transfer time used
  - Optimize the transfer time
- Insufficient exposure time
- If using a CCD imager, the substrate kit was not optimized for the CCD imaging
  - Use the chemiluminescence kit that has strong signal intensity and long signal duration*

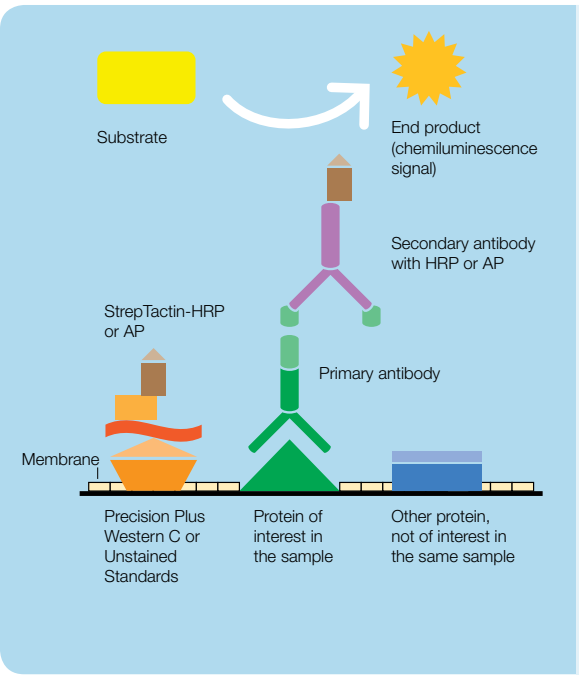


Fig. 1. Mechanisms by which Precision Plus Protein WesternC or unstained standards work with StrepTactin conjugates.

* Such as Immun-Star™ HRP or AP kits or the Immun-Star™ WesternC™ chemiluminescence kit (optimized for CCD imager).

Protein Quantitation Assays — Frequently Asked Questions

For more information, visit **www.bio-rad.com/proteinassays**, or request **Bulletin 1069**.

Introduction

Protein quantitation is an important part of many workflows. The estimation or quantitation of the protein in sample frequently allows researchers to understand the results from their work and make decisions on the subsequent steps. Bio-Rad offers four colorimetric assays for protein quantitation: the Quick Start™ Bradford protein assay, the Bio-Rad protein assay, the *DC*™ protein assay, and the *RC DC*™ protein assay. Knowing which one is the appropriate kit to use and what to watch out for can save time and prevent frustration. Here are the answers to some of the most frequently asked questions related to the usage of these kits.

I want to quantify samples in my protein extraction buffer — which kit should I select?

All Bio-Rad protein assay kits can be used to quantitate samples in common buffers and salts. However, some of the kits such as the Quick Start™ Bradford protein assay or the Bio-Rad protein assay are sensitive to many detergents. The *DC* protein assay can be used to quantitate proteins in the presence of various concentrations of detergents in addition to many common reagents. The *RC DC* protein assay is both reducing agent compatible (*RC*) and detergent compatible (*DC*). To see which kit is compatible with specific contaminating compounds, see Bulletin 1069 or the product manual at **www.bio-rad.com**.

If the protein is in the presence of a compound that has not been tested and this compound can not be removed before quantitation, it would be best to prepare a standard protein dilution series in a similar buffer to determine the effect of the buffer composition on the assay (see Bulletin 1069).

Bio-Rad offers kits with BSA and γ-globulin as control proteins. How do I select a protein standard?

In any protein assay, the best protein to use as a standard is a purified preparation of the protein being assayed. In the absence of such an absolute reference protein, another protein must be selected as a relative standard. The best relative standard to use is the one that gives a color yield similar to that of the protein being assayed. Selecting such a protein standard is generally done empirically. Alternatively, if only relative protein values are desired, any purified protein may be selected as a standard. If a direct comparison of two different protein assays is being performed, the same standard should be used for both procedures. Bio-Rad offers two standards: bovine γ-globulin (standard I) and BSA (standard II). With the Quick Start Bradford and Bio-Rad protein assays, the dye color development is significantly greater with albumin compared to most other proteins, including γ-globulin (Figure 1). Therefore, the BSA standard would be appropriate if the sample contains primarily albumin, or if the protein being assayed gives a similar response to the dye. For a color response that is typical of many proteins, the γ-globulin standard is appropriate. The *DC* and *RC DC* protein assays show little difference in color development between BSA and γ-globulin. It is recommended, however, that the same standard be used if comparisons are to be made between different assays.

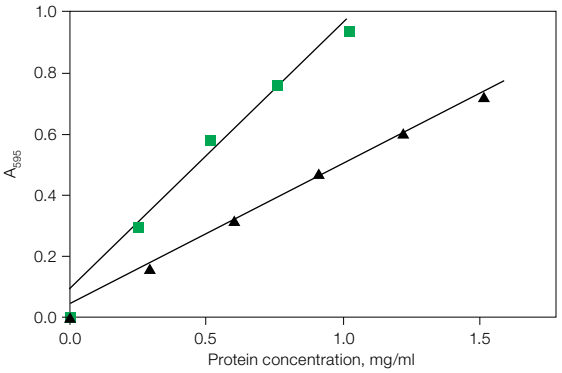


Fig. 1. Typical standard curves for the Bio-Rad protein assay: bovine serum albumin (■) and γ-globulin (▲).

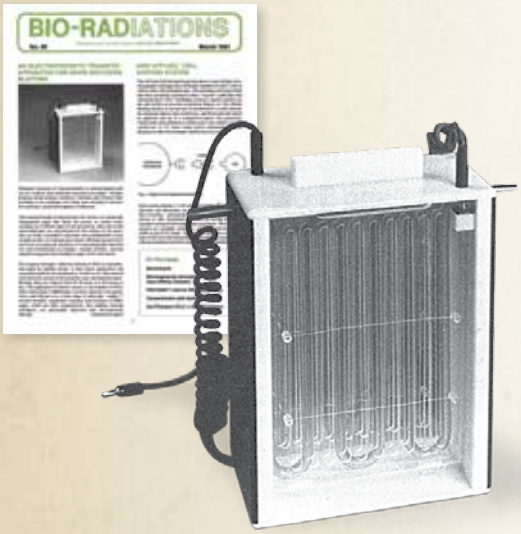
I constructed a calibration curve, but how do I know if my calibration curve is good?

The most common way of constructing calibration curve is fitting the data using linear regression. The quality of the fit of the experimental points to the linear model is usually evaluated using the coefficient of determination R^2. In general, the R^2 should be higher than 0.98 in order to consider the fit of the calibration curve good. Most software applications used to fit the points into a calibration curve can give you the R^2 value.

BioRadiations 1981: An Electrophoretic Transfer Apparatus For Rapid Southern Blotting

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!

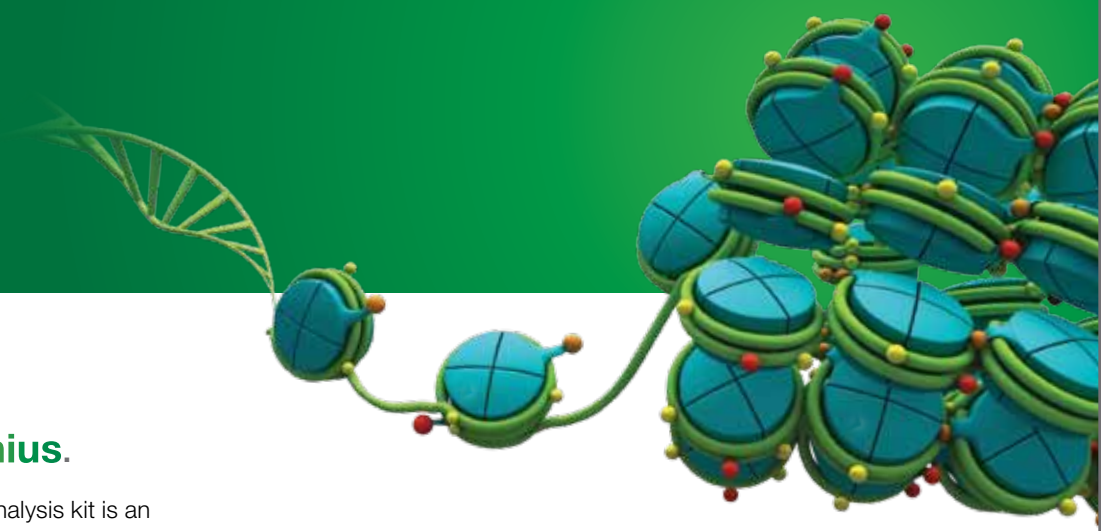


In the mid-1970s, Edward Southern introduced a method for detecting DNA with a hybridization probe. The technique became known as Southern blotting and similar methods subsequently developed for the detection of RNA (northern blotting) and proteins (western blotting) were named as plays on the initial technique. In 1981 Bio-Rad introduced one of the first commercially available apparatuses for rapid Southern blotting — the Trans-Blot® system.

Now!

Today, the Trans-Blot system product line remains a robust part of a comprehensive array of reagents, membranes, and instruments designed to aid in the transfer and detection steps during blotting in the electrophoresis workflow. The latest release, the Trans-Blot® Turbo™ transfer system for western blotting (see page 2) represents next-generation protein transfer by integrating speed, improved performance, and ease of use into a complete system, providing results faster than any other methods currently available.

Go straight to the source of gene expression regulation.



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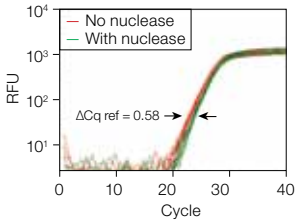
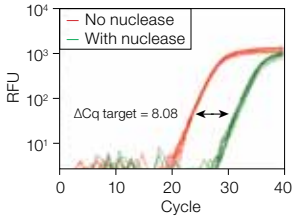


Rhodopsin
Epigenetically silenced

Heterochromatin
0% chromatin accessibility
Minimal Cq shift



GAPDH
Constitutively expressed

Euchromatin
95% chromatin accessibility
Large Cq shift

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