Cover Story
Automating Electrophoresis With the New Experion™ System

Features
Getting Started in Expression Proteomics
Large-Scale Viral Production
Embryonic Stem Cell Transfection

What’s New
High-Fidelity DNA Polymerase
More Reaction Vessels and Sealers
Chromo4™ Real-Time PCR System
Even More Bio-Plex™ Assays
And More …
A Sea of Amplification Options

A complete selection of amplification reagents for both real-time and conventional PCR.

- **Enzymes and kits for a broad array of applications** — hot-start PCR, reverse transcription, and real-time PCR using SYBR Green or probe-based detection
- **High-quality reagents designed for convenience** — easy-to-use kits and protocols simplify reaction setup to generate consistent, reproducible results
- **Products developed and supported by amplification experts** — formulated to provide sensitive detection and linear data over a wide dynamic range

To learn more and enter a drawing to win $500* in amplification reagents, visit us on the Web at [www.bio-rad.com/ad/ampreagents/](http://www.bio-rad.com/ad/ampreagents/)

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* Open to legal US residents only. For a complete listing of sweepstakes rules, visit [www.bio-rad.com/ad/ampreagents/](http://www.bio-rad.com/ad/ampreagents/)

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Denmark 45-44-52-10-00
Finland 358-9-804-22-00
France 33-1-47-95-69-65
Germany 49-89-318-84-0
Greece 30-210-777-4396
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New Zealand 64-9-415-2280
Norway 47-23-38-41-30
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South Africa 27-11-4428508
Spain 34-91-590-5200
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discover.bio-rad.com

On the cover: The Experion electrophoresis station, part of the Experion automated electrophoresis system

in this
BioRadiations 115, 2005

to our readers

Scientific applications of microfluidic technology are rapidly expanding, and have grown to include such traditional, routine applications as electrophoresis. Gel electrophoresis is used daily in laboratories worldwide for basic separations and analyses, including determination of sample composition and purity. Recent automation of the electrophoretic process through application of microfluidics now allows rapid, sensitive, unattended runs, complete with basic sample information. Bio-Rad’s long history of developing products for electrophoresis now includes its introduction of the Experion™ automated electrophoresis system. Some of the product developers describe this exciting new system in this issue’s cover story.

cover story

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**Experion™ Automated Electrophoresis System**

Bio-Rad combines decades of experience in electrophoresis with the innovation of Caliper Life Sciences’ LabChip technology to introduce the Experion automated electrophoresis system, a powerful new system that delivers affordable, accessible automation for protein and RNA electrophoresis to any research laboratory.

**Rapid, Automated Results**

The Experion system applies microfluidic separation technology to speed up data acquisition without compromising data quality. It automatically performs all the steps of gel-based electrophoresis (sample separation, staining, destaining, imaging, band detection, and even some data analysis) to deliver reproducible separation and quantitation results in just 30 minutes.

**Integrated System Design and Superior Performance**

Sleek, user-friendly components exemplify the quality and innovation of the Experion system. Microfluidic chips combine with electrophoresis-grade reagents, automated electrophoresis and priming stations, an easy-to-use vortex station, and powerful analysis software to form an integrated system that streamlines the analytical process.

- Fast, 30 minute batch runs of protein and RNA samples
- Accurate single-step protein sizing from 10 to 260 kD
- Protein resolution and sensitivity comparable to colloidal Coomassie Blue-stained mini gels
- 2-in-1 process for RNA: integrity checks and quantitation
- Optimized reagents and exclusive protein and RNA standards produce accurate, reproducible sizing and quantitation
- Flexible software tools make data analysis easy and efficient

For more information, request bulletin 3140.

**Automation Reduces Hands-On Time Required for Electrophoresis**

The Experion system includes three instruments that automate and expedite the electrophoresis process:

- The electrophoresis station combines the steps of gel-based electrophoresis into a single walk-away process
- The priming station prepares microfluidic chips for electrophoresis — Preset time and pressure settings ensure optimal, consistent introduction of gel solution into the microchannels of the chips, in preparation for sample separation and analysis
- The vortex station ensures sufficient mixing of RNA analysis reagents for effective sample runs

All three instruments have been optimized to minimize user intervention.

**High-Quality Chips and Reagents Ensure Exceptional Resolution and Quantitation**

Experion analysis kits combine innovative chip design with high-quality reagents to perform reproducible, quantitative, and accurate protein and RNA analyses in minutes. Streamlined chip preparation methods and minimal sample requirements result in rapid experiments with minimal hands-on time. Kits are available in flexible ordering configurations to match your research needs.
Each analysis kit includes the following:

- Experion Pro260, RNA StdSens, or RNA HighSens chips
- Optimized gel solution for separation and resolution comparable to results achieved with mini gels
- High-sensitivity fluorescent dye for optimal detection
- Protein or RNA ladder for accuracy in sizing and quantitation
- Sample buffer for optimized, reproducible running conditions

### Specifications for Experion Analysis Kits

<table>
<thead>
<tr>
<th></th>
<th>Pro260 Chip</th>
<th>RNA StdSens Chip</th>
<th>RNA HighSens Chip</th>
<th>Reagents</th>
<th>RNA Ladder</th>
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<tbody>
<tr>
<td>Number of samples</td>
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<td>1–12</td>
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<tr>
<td>Sample volume</td>
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<td>1 μl</td>
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<tr>
<td>Dynamic range</td>
<td>2.5–2,000 ng/μl</td>
<td>5–500 ng/μl</td>
<td>100–5,000 pg/μl</td>
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<tr>
<td>Separation range</td>
<td>10–260 kD</td>
<td>100–6,000 bases</td>
<td>100–6,000 bases</td>
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<td>Sensitivity</td>
<td>2.5 μg/μl of carbonic anhydrase in 1x PBS</td>
<td>5 ng</td>
<td>100 pg</td>
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<td>Shelf life</td>
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<td>Caddy dimensions</td>
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<td>30 x 10 x 50 mm</td>
<td>30 x 10 x 50 mm</td>
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</table>

For more information, request bulletin 3169 and 3170.

### Flexible Software Tools for Efficient Data Analysis

Experion software simplifies your workflow with convenient icons and pull-down menus, and convenient data analysis tools allow you to quickly obtain the information you need. The screen displays separation results in both electropherogram (peak) and simulated gel views. The software automatically calculates protein or RNA component size, concentration, and percent of total sample. Results for each peak appear in the Results table.

- Sizing and quantitation calculations performed automatically
- Intuitive navigation of separation and data analysis screens
- Quick comparisons of protein or RNA components across all samples in the chip
- Multiple printing and data export options

For more information, request bulletin 3171.

For a complete literature packet on the Experion system, request bulletin 3174A.

### Ordering Information

#### Catalog # Description

**Experion Automated Electrophoresis System**

700-7000 Experion System, 100–120/220–240 V, for protein analysis, includes electrophoresis station, priming station, software, USB2 cable, test chip, instructions (analysis kits sold separately)

700-7001 Experion System, 100–120 V, for RNA analysis, includes electrophoresis station, priming station, vortex station, software, USB2 cable, test chip, instructions (analysis kits sold separately)

700-7002 Experion System, 220–240 V, for RNA analysis, includes electrophoresis station, priming station, vortex station, software, USB2 cable, test chip, instructions (analysis kits sold separately)

**Experion Analysis Kits**

700-7101 Experion Pro260 Analysis Kit for 10 Chips, includes 10 Pro260 chips, Experion Pro260 reagents and supplies for 10 chips

700-7102 Experion Pro260 Analysis Kit for 25 Chips, includes 25 Pro260 chips, Experion Pro260 reagents and supplies for 25 chips

700-7103 Experion RNA StdSens Analysis Kit for 10 Chips, includes 10 RNA StdSens chips, Experion RNA StdSens reagents and supplies for 10 chips

700-7104 Experion RNA StdSens Analysis Kit for 25 Chips, includes 25 RNA StdSens chips, Experion RNA StdSens reagents and supplies for 25 chips

700-7105 Experion RNA HighSens Analysis Kit for 10 Chips, includes 10 RNA HighSens chips, Experion RNA HighSens reagents and supplies for 10 chips

700-7106 Experion RNA HighSens Analysis Kit for 25 Chips, includes 25 RNA HighSens chips, Experion RNA HighSens reagents and supplies for 25 chips

Experion LabChip Technology. The Experion analysis chip houses LabChip technology developed by Caliper Life Sciences, Inc. The chip is a powerful, miniaturized device (much like a tiny laboratory) that combines the functionality of several larger benchtop analytical instruments. Up to 10 protein or 12 RNA samples can be analyzed in only 30 minutes. (Chip shown at approximately actual size.)
Model 680 Microplate Reader Updated

The popular Model 680 has been updated to make it the most flexible and versatile microplate reader in the marketplace today. This microplate reader has always been an excellent choice for visible-light colorimetry, with its onboard software, plate-shaking function, 8-position filter wheel, and versatility to read both U- and V-bottom plates and 8- and 12-well strips. The updated version offers even more with these features:

- Clearer LCD display — now 5 mm thicker
- Choice of integrated English and Chinese firmware
- Onboard storage of standard curves and graphs
- Ability to print graphics on external printer — ASCII or ESCIP type formats
- Self-diagnostic capabilities to detect lamp burnout at startup

Ordering Information

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
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<tr>
<td>168-1000</td>
<td>Model 680 Microplate Reader, 100/240 V, 50/60 Hz, includes 415, 450, 490, and 655 nm filters (installed), power cord, spare fuse, dust cover, instructions</td>
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<tr>
<td>168-1001</td>
<td>Model 680 Microplate Reader With Temperature Control, 100/240 V, 50/60 Hz, includes 415, 450, 490, and 655 nm filters (installed), power cord, spare fuse, dust cover, instructions</td>
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<tr>
<td>168-1002</td>
<td>Model 680 Microplate Reader With Internal Thermal Printer, 100/240 V, 50/60 Hz, includes 415, 450, 490, and 655 nm filters (installed), 1 roll of printer paper, power cord, spare fuse, dust cover, instructions</td>
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<td>168-1003</td>
<td>Model 680 Microplate Reader With Temperature Control and Internal Thermal Printer, 100/240 V, 50/60 Hz, includes 415, 450, 490, and 655 nm filters (installed), 1 roll of printer paper, power cord, spare fuse, dust cover, instructions</td>
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<td>168-1100</td>
<td>Model 680 XR Microplate Reader, 100/240 V, 50/60 Hz, includes 340, 405, 415, and 655 nm filters (installed), power cord, spare fuse, dust cover, instructions</td>
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<tr>
<td>168-1101</td>
<td>Model 680 XR Microplate Reader With Temperature Control and Internal Thermal Printer, 100/240 V, 50/60 Hz, includes 340, 405, 415, and 655 nm filters (installed), 1 roll of printer paper, power cord, spare fuse, dust cover, instructions</td>
</tr>
</tbody>
</table>

EXQuest™ Spot Cutter

Rapid Excision With Professional Performance

The EXQuest spot cutter is a next-generation gel excision robot from Bio-Rad. The design includes functionality and value for every laboratory, from occasional precision cutting to high-throughput, multi-gel imaging and cutting. The EXQuest spot cutter is desirable for any laboratory where precision gel cutting and spot identification are required. Benefits include:

- Cutting of over 600 spots/hr
- Illumination for white-light and UV applications
- Cutting of gels and blots
- Full automation of 2-D and 1-D gel cutting
- Walk-away performance
- Precision cutting with 100 µm resolution
- Liquid-handling capabilities for accurate and reproducible performance
- Hands-free processing of up to 4 precast gels
- Streamlined workflow with PDQuest™ 2-D and Quantity One® 1-D analysis software

Ordering Information

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<tr>
<th>Catalog #</th>
<th>Description</th>
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<tr>
<td>165-7200</td>
<td>EXQuest Spot Cutter</td>
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<tr>
<td>165-7201</td>
<td>EXQuest Spot Cutter With PC</td>
</tr>
</tbody>
</table>

The EXQuest spot cutter is fully automated, with a removable gel support tray and four-microplate capacity.
Protein Blotting Starter Kits

The color blot and chemi blot protein blotting starter kits contain all the tools you need for western blotting experiments. The kits contain all materials and reagents required to:

- Transfer proteins to PVDF and nitrocellulose membranes with Tris/glycine transfer buffer
- Block nonspecific sites with nonfat milk
- Wash the membrane and reduce nonspecific binding with TBS and 10% Tween 20
- Label the protein of interest with goat anti-rabbit or goat anti-mouse secondary antibody conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP)
- Detect the protein with your choice of AP- or HRP-mediated chemiluminescent or colorimetric substrate
- Accurately determine molecular weight from blots using unstained or prestained Precision Plus Protein™ standards

The protein blotting guide, included with each protein blotting starter kit, will guide you through western blots from start to finish. This booklet provides information on protein transfer and detection chemistries, methods, equipment, and reagents used in protein blotting. The guide also offers helpful troubleshooting and technical advice. For additional copies of the protein blotting guide, request bulletin 2895.

For more information, request bulletin 3117.

Ordering Information

<table>
<thead>
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<th>Catalog #</th>
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<tr>
<td>170-5050</td>
<td>HRP Color Blot Starter Kit 1, includes 1 L 4CN (HRP substrate), 2 sheets Immun-Blot PVDF membrane, 2 sheets 0.2 μm nitrocellulose membrane, 50 μl Precision Plus Protein™ Kaleidoscope™ standards, 1 L 10x TBS, 100 ml 10% Tween 20, 300 g nonfat dry milk, 2 ml GAR-HRP conjugate, 10x Tris/glycine buffer</td>
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<td>170-5051</td>
<td>HRP Color Blot Starter Kit 2, same as 170-5050 except with 2 ml GAM-HRP conjugate</td>
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<td>170-5052</td>
<td>AP Color Blot Starter Kit 1, includes 1 L BCIP/NBT (AP substrate), 2 sheets Immun-Blot PVDF membrane, 2 sheets 0.2 μm nitrocellulose membrane, 50 μl Precision Plus Protein Kaleidoscope™ standards, 1 L 10x TBS, 100 ml 10% Tween 20, 300 g nonfat dry milk, 2 ml GAR-AP conjugate, 10x Tris/glycine buffer</td>
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<td>170-5053</td>
<td>AP Color Blot Starter Kit 2, same as 170-5050 except with 2 ml GAM-AP conjugate</td>
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<td>170-5054</td>
<td>HRP Chemi Blot Starter Kit 1, includes 100 ml Immun-Star HRP substrate, 2 sheets Immun-Blot PVDF membrane, 2 sheets 0.2 μm nitrocellulose membrane, 50 μl Precision Plus Protein unstained standards, 0.3 ml Precision Protein StrepTactin-HRP conjugate, 1 L 10x TBS, 100 ml 10% Tween 20, 300 g nonfat dry milk, 2 ml GAR-HRP conjugate, 10x Tris/glycine buffer</td>
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<td>AP Chemi Blot Starter Kit 1, includes 100 ml Immun-Star AP substrate, 2 sheets Immun-Blot PVDF membrane, 2 sheets 0.2 μm nitrocellulose membrane, 50 μl Precision Plus Protein unstained standards, 0.3 ml Precision Protein StrepTactin-AP conjugate, 1 L 10x TBS, 100 ml 10% Tween 20, 300 g nonfat dry milk, 2 ml GAR-AP conjugate, 10x Tris/glycine buffer</td>
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<td>AP Chemi Blot Starter Kit 2, same as 170-5056 except with 2 ml GAM-AP conjugate</td>
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Bio-Plex™ Phosphoprotein and Total Target Assay Additions

The latest additions to the Bio-Plex phosphoprotein detection product line include the phospho-p70 S6 kinase, phospho-p90RSK, phospho-STAT2, and phospho-TrkA assays and the total Akt and total p90RSK assays. These easy-to-use bead-based multiplex assays (based on xMAP technology) feature antibodies exclusively developed by Cell Signaling Technology, Inc. They are available individually or as x-Plex™ assays specially premixed and quality-tested at Bio-Rad.

For more information, visit us on the Web at [www.bio-rad.com/ad/phospho/](http://www.bio-rad.com/ad/phospho/)

### Available Phosphoprotein and Total Target Assays

<table>
<thead>
<tr>
<th>Phosphoprotein assays</th>
<th>Total target assays</th>
<th>Phosphorylation site</th>
<th>Bead region</th>
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<tr>
<td>Akt</td>
<td>ATP-2</td>
<td>EGFR</td>
<td>ERK1</td>
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<td>Phosphoprotein assays</td>
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<td>Total target assays</td>
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<td>Phosphorylation site</td>
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<td>Bead region</td>
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* This assay cannot be multiplexed.

### Ordering Information

Catalog # | Description
--- | ---
171-304004 | Bio-Plex Phosphoprotein Detection Reagent Kit, 1 x 96-well
171-304005 | Bio-Plex Phosphoprotein Detection Reagent Kit, 10 x 96-well
171-304011 | Bio-Plex Cell Lysis Kit, 1 x 96-well
171-304012 | Bio-Plex Cell Lysis Kit, 10 x 96-well
171-V21075 | Bio-Plex Phospho-Akt (Ser473) Assay, 1 x 96-well
171-V21620 | Bio-Plex Phospho-ERK1 (Thr202/Tyr204) Assay, 1 x 96-well
171-V21938 | Bio-Plex Phospho-ERK2 (Thr180/Tyr182) Assay, 1 x 96-well
171-V22238 | Bio-Plex Phospho-ERK1/2 (Thr202/Tyr204, Thr180/Tyr182) Assay, 1 x 96-well
171-V2318 | Bio-Plex Phospho-GSK-3α/β (Ser21/Ser9) Assay, 1 x 96-well
171-V20758 | Bio-Plex Phospho-IκB-α (Ser32/Ser36) Assay, 1 x 96-well
171-V21034 | Bio-Plex Phospho-JNK (Thr183/Tyr185) Assay, 1 x 96-well
171-V21336 | Bio-Plex Phospho-p38 MAPK (Thr180/Tyr182) Assay, 1 x 96-well
171-V23318 | Bio-Plex Phospho-STAT2 (Tyr689) Assay, 1 x 96-well
171-V22552 | Bio-Plex Phospho-STAT3 (Tyr705) Assay, 1 x 96-well
171-V23973 | Bio-Plex Phospho-TrkA (Tyr490) Assay, 1 x 96-well
171-V21034 | Bio-Plex Phospho-JNK (Thr183/Tyr185) Assay, 1 x 96-well
171-V21336 | Bio-Plex Phospho-p38 MAPK (Thr180/Tyr182) Assay, 1 x 96-well
171-V23232 | Bio-Plex Phospho-STAT2 (Tyr689) Assay, 1 x 96-well
171-V23535 | Bio-Plex Phospho-p90RSK (Thr202/Ser204) Assay, 1 x 96-well
171-V31075 | Bio-Plex Total Akt Assay, 1 x 96-well
171-V31620 | Bio-Plex Total ATF-2 Assay, 1 x 96-well
171-V30438 | Bio-Plex Total ERK2 Assay, 1 x 96-well
171-V30758 | Bio-Plex Total IκB-α Assay, 1 x 96-well
171-V31336 | Bio-Plex Total p38 MAPK Assay, 1 x 96-well
171-V32552 | Bio-Plex Total STAT3 Assay, 1 x 96-well
171-V33535 | Bio-Plex Total p90RSK Assay, 1 x 96-well

### Double Ligand Screen Data for Cytokine Release From Macrophages

Now Available From the Alliance for Cellular Signaling

Cytokines and chemokines are essential outputs of receptors and signaling networks in macrophages and are critical regulators of inflammatory and immune responses. The Alliance for Cellular Signaling (AfCS) has conducted a comprehensive ligand screen consisting of time courses of RAW 264.7 cells exposed to 22 ligands individually and to each of their possible pair-wise combinations. The results, generated by liquid suspension array (Bio-Plex™ system for analysis of 18 cytokines), confirm known pathways of cytokine production and reveal novel regulation and interactions between signaling pathways. The cytokine data, which are available for the research community to mine and interpret freely, will be integrated by the AICS with other initiatives (transcriptional microarrays, cAMP, calcium, phosphoproteins, lipids, and RNAi perturbations) to help define and model the complex signaling networks and molecular interactions that regulate cytokine production during infection and inflammation.

You can access the data at [www.signaling-gateway.org/data/cgi-bin/table2.cgi?cellabbr=RW](http://www.signaling-gateway.org/data/cgi-bin/table2.cgi?cellabbr=RW)
A World of Information

Achieve unsurpassed multianalyte detection and quantitation with the integrated Bio-Plex suspension array system.

Quantitate up to 100 different analytes in a single microplate well.
Multiplexing with the Bio-Plex system yields interlinked data within a biological system so that complex relationships and pathways of biomolecules can be easily revealed.

For more information, visit us on the Web at www.bio-rad.com/info/bio-plex/
iProof™ High-Fidelity DNA Polymerase

PCR polymerases often have provided either high fidelity or high processivity, but not both. Using patented* Sso7d fusion technology, Bio-Rad has incorporated both of these valuable PCR parameters into a single enzyme, iProof high-fidelity DNA polymerase. Now you don’t have to sacrifice either fidelity or processivity when performing PCR, which allows you to accurately and efficiently amplify a wide range of DNA templates for use in a variety of molecular biology applications.

- **Fidelity** — the most accurate thermostable polymerase available (52-fold more accurate than Taq)
- **Speed** — dramatically reduces extension steps and overall reaction times (15–30 sec/kb)
- **Length** — amplifies large fragments (up to 37 kb) in less time and with less enzyme (0.25–1 unit/reaction)

iProof high-fidelity DNA polymerase delivers unsurpassed speed and fidelity, with higher yields and fewer reaction failures. It is available in three formats: a stand-alone enzyme, an easy-to-use master mix, and a PCR kit with controls.

For more information, request bulletin 5211 or visit us on the Web at www.bio-rad.com/iproof/

**Ordering Information**

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<td>172-5301</td>
<td>iProof High-Fidelity DNA Polymerase, 100 U</td>
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<td>172-5311</td>
<td>iProof HF Master Mix, 500 reactions (for highest fidelity with most templates)</td>
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<tr>
<td>172-5321</td>
<td>iProof GC Master Mix, 500 reactions (for GC-rich templates)</td>
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<tr>
<td>172-5331</td>
<td>iProof High-Fidelity PCR Kit, 200 U</td>
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* US patent 6627424 and patents pending
Expanded Offering of Reaction Vessels and Sealers

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Many real-time thermal cyclers require an internal reference dye for fluorescent signal normalization and correction of well-to-well optical variations. ROX passive reference dye allows seamless integration of non-ROX-containing PCR reagents on all ROX-dependent real-time instrument systems.* An internal reference is not required for use with the iCycler iQ® or MyiQ^® real-time detection systems, or the MJ Research® Opticon® or Chromo4® detectors.

**Ordering Information**

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
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<tbody>
<tr>
<td>170-8858</td>
<td>ROX Passive Reference Dye, 0.5 ml</td>
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* Since excitation optics vary on different instrument platforms, the optimal reference dye concentration must be matched to the given real-time cycler. This dye has been formulated as a 50x concentrated stock solution for use on the ABI PRISM 7000, 7300, 7700, or 7900 systems. For instruments employing a 580–585 nm excitation for the passive reference, such as the Stratagene Mx4000, Mx3000P, or Mx3005P, or the ABI PRISM 7500 sequence detection system, the ROX passive reference dye should be treated as a 750x concentrated solution.
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Delivery of siRNA in MCF-7 cells. Cells were transfected with 10 nM siGLO siRNA using 0.5 μl siLentFect. After 24 hr, cells were imaged to show nuclear staining by Hoechst 33342 dye (top) or the location of fluorescent siRNA (bottom).
what's new

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- Interchangeable Chromo4 detector fits all standard DNA Engine base units
- Powerful DNA Engine thermal cycler offers precise thermal control and a temperature gradient feature

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Ordering Information
Catalog # Description
CFD-3240 Chromo4 Continuous Fluorescence Detector, includes optical module and analysis software
CDM-3240-01 Chromo4 Photonics Shuttle, contains LEDs, photodiodes, and filters
ALS-3296 96-Well Alpha Unit for Chromo4 Detector

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Both lipids provide exceptional performance in the absence or presence of serum-containing media at higher cell culture densities. The streamlined protocol allows you to transfect cells with little loss in efficiency or overall reporter gene activity.

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- Rapid transfection — transflect cells as they are plated
- Low cytotoxicity — ensure high levels of expression
- Flexible protocols — transfect in the presence or absence of serum

HEKFectin has been tested and validated using HEK 293, 293H, and 293F cells. COSFectin has been tested and validated using COS-7 cells.

Ordering Information
Catalog # Description
170-3370 COSFectin Cell-Specific Lipid, for the transfection of COS cells and subclones, 0.5 ml
170-3371 COSFectin Cell-Specific Lipid, 1.0 ml
170-3372 COSFectin Cell-Specific Lipid, 5 x 1.0 ml
170-3380 HEKFectin Cell-Specific Lipid, for the transfection of HEK 293 cells and subclones, 0.5 ml
170-3381 HEKFectin Cell-Specific Lipid, 1.0 ml
170-3382 HEKFectin Cell-Specific Lipid, 5 x 1.0 ml

β-Galactosidase expression in mammalian cell lines transfected using Bio-Rad and other commercially available cell line-specific lipids. Cell cultures were transfected with pCMV/SPORT-β-gal. After 24 hr, cell extracts were assayed for β-galactosidase activity. Top, COS cells; bottom, HEK 293 cells. Controls were untransfected cells.

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Expression proteomics, or comparative proteomics, contributes to our understanding of biological systems by comparing protein profiles of different cell states (for example, normal vs. diseased in order to rapidly detect new biomarkers for diagnosis and treatment of various diseases). In adopting a strategy for comparative proteomics, there are several issues that must be considered. We highlight some of the major issues here.

**2-D PAGE-MS vs. LC-MS**

Protein separation and quantitation can be accomplished by two complementary methods, either two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) or multidimensional liquid chromatography (LC); both approaches are then followed by mass spectrometry (MS) (Figure 1). As more laboratories start up their own proteomic efforts, they have to decide which path they should follow first. Unfortunately, there is no simple answer to this question, due to the fundamental differences each approach exhibits. This has been demonstrated by Schmidt et al. (2004), who performed analyses of the *Mycobacterium tuberculosis* proteome using both 2-D PAGE-MS and LC-MS, the latter using an isotope-coded affinity tag (ICAT) to enhance the limited quantitative ability of MS. Interestingly, only 7% of all 388 proteins identified were detected by both procedures.

The two methods provide complementary information. LC-MS, for example, shows a clear bias for high molecular weight proteins, whereas 2-D PAGE-MS shows a preference for low molecular weight proteins. Most importantly, 2-D PAGE-MS differentiates between posttranslationally processed and unmodified polypeptides, while LC-MS can only quantitate all protein species of one gene product.

In addition to these fundamental differences, several other considerations may lead researchers to initially adopt a 2-D PAGE-MS approach before pursuing an LC-MS approach. Electrophoresis, the core technology used in 2-D PAGE-MS and a common technique in daily lab work, results in a shorter learning curve for the 2-D gel approach. In addition, based on the vast amount of data produced from LC-MS spectra versus the data produced from a 2-D gel run, data presentation from a 2-D data set presents a faster result to interpretation. Finally, 2-D PAGE is one of the most cost-effective ways to begin comparative proteomics research. With reasonably priced electrophoresis equipment and the availability of MS outsourcing, the need for investment in mass spectrometry can be delayed.

The main advantage to LC-MS is its amenability to automation, which is important for large-scale proteomics studies. Chromatography itself presents numerous ways to separate peptides, including reverse phase, ion exchange, size exclusion, or affinity methods (Liebler 2002). Additionally, LC-MS is able to represent protein classes that 2-D PAGE-MS is known to underrepresent, such as very acidic or basic proteins and extremely large or small proteins (Peng and Gygi 2001).

Although there are pros and cons for both the 2-D PAGE-MS and LC-MS approaches, the gel-based approach is still considered the workhorse of proteomics and will continue to be an integral part of proteomics for the foreseeable future. The success of gel-based proteomics (2-D PAGE-MS), however, relies on careful consideration of several key issues. These key issues are outlined below.

---

**Fig. 1. Complementary expression proteomics workflow.** Upper path, 2-D PAGE-MS; lower path, LC-MS.
Sample Preparation
Sample preparation dramatically affects both reproducibility and resolution of 2-D gels. Common gel artifacts, such as streaking and smearing, can be reduced by a general cleanup of the sample prior to isoelectric focusing (IEF). A variety of options exist for general cleanup of protein samples, including spin-column purification, protein precipitation, and total protein extraction, to name a few (see bulletin 3096).

Alternatively, the complexity of crude protein samples can be reduced by fractionation during sample preparation. The proteome of a human cell has been estimated to have up to 20,000 protein species (Celis and Gromov 1999). 2-D gels, at best, can resolve only 3,000 protein spots. Fractionation enables enrichment of low-abundance proteins that might not otherwise be visualized (Figure 2). To push the limits of gel-based expression proteomics, a variety of powerful fractionation tools (reagents and instruments) are available to reduce sample complexity (see bulletin 3096).

Resolution
Four areas warrant attention to optimize the resolving power of 2-D PAGE-MS. The first area is the efficient and reproducible extraction and solubilization of proteins. One of the major causes of streaking and background smearing is poor protein solubilization, or insufficient solubility of proteins during isoelectric focusing (IEF). Special attention should be paid to the solubilization buffer formulation to solubilize as many proteins as possible while maintaining their solubility throughout 2-D PAGE (Berkelman et al. 2004). The best combination of chaotropic agents, detergents, and reducing agents must be determined empirically for each protein sample.

The second area relates to sample preparation. Resolution is compromised by contaminants that negatively impact IEF, SDS-PAGE, or both, and cause either horizontal or vertical streaking. Contaminants of this nature include salts, ionic detergents, small compounds, nucleic acids, lipids, phenolic compounds, and polysaccharides.

The third and fourth areas are the IPG-strip characteristics (length and pH interval) chosen for isoelectric focusing, and the size of the second-dimension gel, respectively. Wide IPG gradients, such as from pH 3 to 10, offer an overview of the proteome, while multiple, overlapping micro- or narrow-range IPG strips are useful for in-depth analysis. A larger second-dimension gel allows better separation between adjacent spots (which may overlap on smaller gels), yielding more individual protein spots per gel.

Protein Staining
Dyes used for visualization of gel-separated protein spots must be highly sensitive, provide accurate quantitative data, and be compatible with mass spectrometry. Traditionally, 2-D gels have been stained either by silver or colloidal Coomassie Blue, but have a lower dynamic range ($<10^5$), limiting protein quantitation. These two stains are better used as screening tools or for optimization purposes when protein quantitation is not critical.

In contrast, fluorescent dyes such as SYPRO Ruby offer the highest sensitivity (~1 ng of protein per spot) over a broad dynamic range ($10^4$), which ensures excellent reproducibility. Fluorescent dyes also enable multiplexed proteomic analysis (Patton 2002). For example, glycosylated and phosphorylated proteins can be resolved by probing a single gel with fluorophores that have different excitation and/or emission maxima. Table 1 lists staining and compatibility characteristics of the most widely used dyes, including recommended equipment for imaging and compatibility with mass spectrometry.

Reproducibility
Reproducibility is probably the biggest concern among scientists performing 2-D PAGE-MS, who may regard it as a labor-intensive procedure that still results in poor data quality, especially for newcomers trying to establish this method in their...
labs. However, major advances in standardization, and hence in reproducibility, have come from commercially available premixed electrophoresis buffers, batch-prepared sample preparation kits, ready-made gels and IPG strips in various sizes and formats, optimized staining reagents, high-resolution scanners, and sophisticated software. Additionally, dedicated and easy-to-use instruments like the PROTEAN® IEF cell, the Dodeca cells (Zhan and Desiderio 2003), and the Dodeca stainers allow running and staining of up to 12 samples in parallel. Used together, these tools improve data quality tremendously.

Even with all these tools, statistically reliable data must include gel replicates for each sample. Three to four replicate gels per sample are usually statistically sufficient to exclude false positives, even within the limit of 1.5–2.0-fold change normally associated with 2-D gel experiments. The standard deviation in a replicate group of three gels obtained from one sample and within a single 2-D PAGE run is usually 10–20% for the vast majority of spots (Blomberg et al. 1995). Using three replicate gels per sample as a rule of thumb allows comparison of identical cell lines or similar bacterial strains. However, when comparing heterogeneous samples such as human biopsies, the data must be validated by in-depth statistical analysis, and the number of gels required may be greater than three (Dr Stuart Cordwell, Australian Proteome Analysis Facility, personal communication). Sophisticated data analysis packages, like PDQuest™ 2-D analysis software, offer a variety of simple-to-use analysis tools for data evaluation and integration. These tools make it easy to draw meaningful conclusions from high-quality, reproducible 2-D PAGE results.

**Summary**

Comparative proteomics requires evaluating many key considerations before deciding on the optimal path. No single set of techniques addresses every concern. However, 2-D PAGE-MS represents a powerful and flexible method that enables analysis of proteins in complex systems for a reasonable up-front investment. Though not flawless, 2-D PAGE-MS meets more than the fundamental needs for the initial and later stages of proteome research, and it will be constantly improved to meet the demands of the future. If your decision leads you to pursue gel-based proteomics, we have highlighted considerations that should help ensure success. Be sure to properly prepare and solubilize your sample, choose appropriate gel and IPG-strip characteristics, use appropriate protein stains, minimize variation among runs, and use appropriate statistical tools. For more information on expression proteomics, or for 2-D PAGE troubleshooting advice, visit Bio-Rad’s expression proteomics web site at [www.expressionproteomics.com](http://www.expressionproteomics.com)

**References**


Merril CR et al., Trace polypeptides in cellular extracts and human body fluids detected by two-dimensional electrophoresis and a highly sensitive silver stain, Proc Natl Acad Sci USA 76, 4335–4339 (1979)


Excision Express

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Get on board the Excision Express and realize precision gel cutting for accurate identification by mass spectrometry. With the EXQuest spot cutter, you can walk away and reliably pick up and deliver spots from visible- or UV-stained gels of any size, backed or unbacked, at 600 spots per hour. The affordable ticket to hands-free imaging and cutting gives you increased throughput — cut up to four gels at a time — along with multiplate delivery. Only the EXQuest spot cutter offers all this plus true software integration with 1-D and 2-D analysis software, for the ultimate in nonstop convenience.

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The Experion™ System

Bio-Rad Applies Microfluidics to Automate Gel Electrophoresis

Transforming Gel Electrophoresis

For decades, scientists have gained essential data about their protein and nucleic acid samples from the use of gel-based electrophoresis. Electrophoresis, the movement and separation of charged particles in response to an electric field, results in unparalleled resolution of biomolecules. However, the process is composed of a series of manual steps that require numerous pieces of equipment, various reagents, and several hours to gain the information needed from sample separations. Since this traditional process requires significant time and manual labor, automation has been a key desire of scientists. Bio-Rad’s new Experion system applies innovative microfluidic separation technology to both automate and accelerate this process.

Authors: Blanca Chang, Eric Larson, and Christina Whitman-Guliaev
Bio-Rad Laboratories, Inc., Hercules, CA USA
The growing use of microfluidics technology is very promising for many scientific applications. The Experion system implements and tailors this technology to gel-based electrophoresis, offering fast, accurate, high-resolution separation and analysis of protein and RNA. The system requires only very small amounts of sample for analysis, allowing fast decision making in experiments that rely on protein or RNA assessments. Because of the many downstream applications that rely on separation and analysis of protein and RNA, this system presents significant advantages to the research scientist. This article introduces the technology of microfluidics, Bio-Rad’s system approach, and the advantages of using the Experion system for research applications.

Microfluidic Separation Technology

The term “microfluidics” refers to the transport and manipulation of very small volumes of liquid. Generally, microfluidics includes not only miniaturization of the channels that the liquid flows through but also the movement and interaction of materials of interest — dissolved or suspended in the liquid — by fluid dynamic, electrophoretic, and chemical principles on a small scale. Microfluidic separation technology brings the advantages of miniaturization and automation to a variety of different chemical and biochemical analyses. These analyses benefit from dramatically reduced time to results, reagent and sample consumption, and user intervention.

Developed by Caliper Life Sciences, Inc., LabChip microfluidic chips perform sample separations within tiny chambers and channels that are fabricated in quartz, glass, or plastic. As with electronic devices, these microchannels and microscopic chambers may serve different functions within a single chip. This allows the integration of several different processes, such as performing sample and reagent dilution, mixing, incubation, and separation all on a single chip. It also enables the introduction and analysis of multiple samples. The microchannels are generally 50 µm wide and 10 µm deep, somewhat smaller than a strand of human hair, and these small dimensions reduce both processing times and the amounts of sample and reagent required for an assay. Microfluidic circuits, coupled with the appropriate software and instrumentation to control separation, detection, and analysis, can be designed to combine the functionalities of several larger benchtop analytical instruments. Depending on their design, these circuits can accommodate virtually any analytical biochemistry process, including separation of nucleic acids and proteins by electrophoresis.

LabChip microfluidic chips combine the semiconductor manufacturing processes used in the electronics industry with wet-chemistry laboratory techniques. Designing the optimal configuration and dimensions of microchannels and chambers is a complex process requiring the application of fluid dynamics, biophysics, and principles of biochemistry and chemical and electrical engineering. Microfabrication manufacturing methods are used to produce the small, precise, and intricate patterns of interconnected channels in the Experion chips. First, a photolithographic process is used to etch the channel circuit designs into sheets of glass. Another sheet is then bonded to the first to cover the channels and convert them into closed microfluidic circuits. The end of each channel is connected to an open reservoir through which fluids can be introduced. Individual chips are then packaged into plastic holders, called caddies, to facilitate handling. These microfabrication methods are highly reproducible and are capable of generating large quantities of chips in a relatively short amount of time.

Electrokinetic forces can be used to direct the movement of molecules through microfluidic
separation devices. Electrokinetic forces result when electrodes are attached to power supplies and placed into reservoirs at each end of the microchannel, generating an electrical current. Charged molecules in solution move and are separated by electrophoresis. For the electrophoresis process, the microchannels are filled with a gel solution to accomplish the desired separation. The gel solution also eliminates electroosmosis to produce reproducible separation results.

The chips are an integrated part of the microfluidic system, working together with specialized computer-controlled instrumentation used to manipulate the timing and sequence of the processes designed into the chip. The low velocities and small channel dimensions of microfluidic systems lead to predictable motion of fluids and charged molecules. As a result, these systems inherently generate accurate, reproducible data. Nevertheless, chip design must provide optimal channel and sample well dimensions, and system design must provide precise control over temperature, flow rates, and sample injection and separation voltages, to name a few, in order to produce optimal results.

**Electrophoresis With the Experion System**

The Experion system applies Bio-Rad’s decades of knowledge and experience in protein and nucleic acid separations to LabChip microfluidic technology. LabChip technology is the leading commercial application of microfluidic principles to laboratory science. With Bio-Rad’s contribution of reliable electrophoresis equipment, reagents, standards, and gel solutions, the Experion system provides the performance that is required to obtain meaningful data in a laboratory setting.

When microfluidic chips are used for electrophoresis, the process is, overall, very similar to that of traditional gel-based electrophoresis. The main difference is that microfluidics enables the miniaturization and combination of multiple steps of gel-based electrophoresis — separation, staining, destaining, imaging, and even basic data analysis — into a single automated process.

The Experion microfluidic chip is the entry port for sample separation and detection. To prepare the chip for separation, gel solution is introduced into the microchannels by a pressure-driven process called “priming”. Once the chip has been primed, samples are loaded into the appropriate wells for separation. A preprogrammed series of voltage changes applied at the sample wells allows small amounts of sample to be directed from one channel and injected into another. As a sample moves into a separation channel, its individual sample components begin to separate. In the presence of gel, electroosmotic forces are suppressed and electrophoretic mobility acts as the sole mechanism of sample movement and separation during the entire analysis process.

A fluorescent dye is incorporated into the sample components during separation. As the sample components pass through the separation microchannel, they are detected by laser-induced fluorescence as they pass by the position of a focused laser. The fluorescence and migration time data are transmitted to the software, which presents them in both electropherogram and simulated gel views (see Protein Analysis sidebar). Software analysis tools simultaneously perform molecular weight sizing, peak detection, or both, as well as automatic quantitation of the individual peaks.

The Experion system components — the microfluidic chip, reagents, instrumentation, and software — combine to accomplish separation, staining, destaining, detection, quantitation, and basic data analysis with minimal user intervention in a fraction of the traditional time. The run times for samples applied to a single chip (10–12) are only 30 minutes using the Experion system.

**Major Advantages**

**Rapid, Automated Results**

Bio-Rad’s Experion system performs the multiple steps of gel-based electrophoresis with automated ease and speed, while generating highly resolved results. It performs reproducible separations of up to 10 protein or 12 RNA samples in just 30 minutes, including data analysis, significantly reducing time to results compared to traditional methods. The unattended process frees up time to improve lab efficiency.

**Low Sample Requirements**

With detection capabilities down to low nanogram and even picogram amounts of sample, and volume requirements of just 1–4 µl, the microfluidic format enables the Experion system to use a very small amount of sample for separation and analysis.
This is especially important in cases where a precious sample is in short supply, or must last over many experiments such as for RT-PCR or microarray experiments. The Experion system not only provides a uniquely comprehensive view of the quality and concentration of the sample, but also requires only a small amount of sample and time, making it an ideal checkpoint for successful completion of gene expression studies.

Familiar Data Output
The Experion system displays separation data in formats that are familiar and easy to use. Each sample separation is displayed as an electropherogram, similar to a chromatogram, and as a simulated gel image (see sidebars). This approach provides optimal information and a convenient means of analysis. The electropherogram is an excellent view for analyzing small peaks among

Experion System Tour

Instrumentation
The Experion electrophoresis station is the comprehensive sample running apparatus, power supply, and detector, all built into one unit. Electrophoresis occurs by delivering voltage to each well in an analysis chip. The electrode manifold is carefully engineered to line up 16 platinum electrode pins with the 16 wells in a chip. The electrophoresis station is built to provide consistent separation performance while remaining an affordable benchtop apparatus.

Eliminating the most likely source of error, incorrect or inconsistent priming, the automated priming station is designed with preset pressure and time settings that are optimized for the different Experion analysis chips. A “priming code” printed on each chip is used for setting the priming station pressure and time. A unique automatic release stops priming at the end of the timed cycle, which lasts 30–60 seconds, depending on the analysis chip. This provides highly consistent chip priming without the need to monitor and manually stop the priming cycle. Because of the viscosity of the gel solution, ambient temperature can have a significant effect on the priming. The Experion priming station has a unique temperature sensor and automatically adjusts the pressure to compensate for the temperature, ensuring reproducible results. These features combine to consistently deliver the right amount of pressure run after run, ensuring error-free automated priming.

For effective separation and analysis of RNA samples, samples and reagents must be mixed in the chip prior to separation. This is achieved with the vortex station, which is designed with preset speed and time settings for precise mixing of samples and reagents for effective sample runs. The specially designed vortex adaptor provides easy chip access and securely holds the chip during the 1 minute vortex cycle.

Analysis Kits
The Experion analysis kits include chips and reagents that are optimized for electrophoresis of particular sample types (protein or RNA). The analysis kits include standards to allow quantitation and sizing of the sample components. Two chip and reagent kit designs are available for RNA analysis, the Experion RNA StdSens kit for RNA detection at the nanogram level, and the RNA HighSens kit for detection at the picogram level. The Experion Pro260 kit is used for protein separation, molecular weight sizing from 10 to 260 kD, and quantitation of protein samples.

Software
The Experion software controls the voltages applied by the electrophoresis station for sample injection and separation, analyzes fluorescence data from the detector, and provides a display of the separation results. The software clearly displays extensive information, including sizing, quantitation, and visual comparisons of sample components, on a single screen. Additional analysis functions allow the flexibility to quickly sort and compare information within the chip. For example, a specific protein peak can be selected and its characteristics compared across the wells of the chip. Electrophoresis is a powerful resolving tool and, like a gel or chromatogram, the Experion data display is an informative tool for reference. Various reports can be generated, either by printing or exporting the data in image or table format. The Experion software offers a powerful combination of the resolving power of a gel with the ease of digital data access.

For more information on individual components of the Experion system, see the What’s New article on pages 2–3.
many larger peaks or for close examination of the resolution of two very closely migrating peaks. The gel image is ideal for performing sample comparisons. Furthermore, automatic calculations are displayed in Results tables for improved user convenience.

**Automatic Sizing and Quantitation**

The Experion system automatically determines the sizes and quantities of protein and RNA sample components. The Protein Analysis and RNA Analysis sidebars provide more detail about how sizing and quantitation are accomplished.

The results of the automatic sizing and quantitation calculations are conveniently displayed in the Results table of the software screen. In protein samples, information is displayed for each detected protein peak, including the molecular weight, quantity, and percent of the total sample. Further user-directed analysis of one or more specific proteins can be accomplished using the Protein table, which allows the comparison of selected protein(s) across all the samples of a chip. The Proteins tab presents the results with a summary of the mean, standard deviation, and coefficient of variation.

For RNA samples, the Results table displays the total quantity of the sample. In a total RNA run, the areas of the ribosomal peaks are shown and the areas used to calculate the ribosomal RNA ratio, which is a standard indication of RNA integrity. For mRNA samples, the amount of ribosomal contamination is reported along with the total sample quantity.

**Conclusions**

The Experion system advances electrophoresis automation through the combination of microfluidic technology and electrophoresis expertise. It expands the researcher's ability to produce data quickly, without compromising the quality of results. This affordable, innovative system offers exceptional sensitivity, resolution, and data analysis for protein and RNA samples. In addition, the Experion system offers a variety of advantages over traditional gel electrophoresis, such as faster results, reduced sample and reagent usage, automatic calculations, reduced hands-on time, and elimination of the challenges, tedium, and hazardous materials typically associated with these applications. By automating sample separation and analysis and freeing up researcher time, this integrated system expedites research goals, leading to faster results in a wide variety of applications. It can also be used as a complementary tool to many of the most important and common downstream laboratory experiments that require knowledge of the quality and composition of protein or RNA samples.
**RNA Analysis**

The Experion system is designed for rapid analysis of RNA samples in either picogram or nanogram ranges. Each Experion RNA analysis kit contains the chips and reagents required to perform RNA electrophoresis. The RNA HighSens kit accommodates 1–11 samples, and the RNA StdSens kit accommodates 1–12 samples; both kits are available in 10-chip and 25-chip configurations.

The RNA analysis kits include an RNA ladder that is specifically produced for Experion system applications. The quality and accuracy of the individual RNA transcripts are tested before blending to create a precise ladder, resulting in accurate quantitation of RNA samples. The RNA analysis kits also include loading buffer that contains an internal lower marker, which serves to normalize the separation of the sample to the separation of the RNA ladder.

When analyzing mRNA samples, it is important to determine the extent of rRNA contamination. Contaminating RNA can be detected, along with its concentration, using Experion RNA analysis kits. Separation generates a broad mRNA sample peak, and any contaminating rRNA peaks will be visible. rRNA peaks and RNA ratios, such as 28S:18S, are calculated for total RNA samples.

The Experion system offers a 2-in-1 system for RNA analysis. It performs both automatic calculations of quantity and a qualitative visual assessment of the RNA sample in a single step. The results are displayed in an electropherogram and simulated gel view, which indicates if the sample has been degraded or has extensive DNA or rRNA contamination. Thus, the Experion system obviates the need to run a gel for visual assessment or to take spectrophotometer readings for determination of concentration.

**RNA Workflow**

- **RNA Purification and Analysis for Differential Gene Expression**
  - RNA Isolation and Purification
  - RNA Purity or Quality
  - Determination of RNA Concentration
  - Real-Time PCR
  - DNA Microarray Analysis

**RNA Analysis**

- Total RNA (With RNA StdSens Kit)
- mRNA (With RNA HighSens Kit)

**Protein Workflow**

- **Protein Analysis for Drug Discovery**
  - Protein Purification
  - Protein Sizing
  - Determination of Protein Concentration
  - Statistical Comparisons
Feasibility of Multiplexing Bio-Plex™ Total Target and Phosphoprotein Assays

Qian Gao, Joella Blas, and Efthalia Gerasimopoulos, Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction
Bio-Plex assays allow detection of multiple target proteins in a small volume of sample. The assays are based on detection of signal from tagged fluorescent beads (xMAP technology) that have bound to each target protein. Typically, each type of bead is conjugated with distinct antibodies that recognize one of the target proteins. Appropriate sets of beads are then selected that enable simultaneous, or multiplex, assays of the proteins of interest in the sample. Bio-Plex phosphoprotein assays report the level of a protein that is phosphorylated at a specific site or sites, while total target assays report the level of the protein regardless of its phosphorylation state.

Methods
We designed the study using HEK 293 cells, which respond to UV treatment by increasing the level of phosphorylation of several proteins. All proteins selected for the phosphoprotein assays in this study are activated by this treatment. The multiplex run (6-plex) included the following targets:

- Total ERK2
- Phospho-p53 (Ser15)
- Phospho-p70 S6 kinase (Thr421/Ser424)
- Phosho-ATF-2 (Thr71)
- Phospho-JNK (Thr183/Tyr185)
- Phospho-c-Jun (Ser63)

Untreated and UV-treated HEK 293 cell lysates were prepared according to methods detailed in the Bio-Plex phosphoprotein assay instruction manual. Both untreated HEK 293 and UV-treated HEK 293 cell lysates were tested at three different protein concentrations to test dose-response characteristics of the assays. Blank wells were included in the run, with cell lysis/assay buffer used to replace the cell lysate. Duplicates were run for each sample.

Results and Discussion
Total Target Assays Can Be Multiplexed With Phosphoprotein Assays for Distinct Targets
Data from a multiplex run of five phosphoprotein assays with a total ERK2 assay are presented in Figure 1. To show the results clearly, each phosphoprotein signal was plotted together with the total ERK2 assay signal. Although the phosphorylation levels found between UV-treated and untreated cell lysates varied, the total ERK2 signal level at the same protein concentration was very consistent between treatments, indicating that none of the phosphoprotein assays interfered with the total target assay in this multiplex run.

For all tested phosphoprotein targets, the level of detected phosphorylation increased in HEK 293 cells in response to UV treatment, with the extent of increase depending on the phosphoprotein, as expected. The most dramatic signal increase was detected for phospho-c-Jun (Ser63) (Figure 1E). At 0.6 µg/ml protein, there was more than a 40-fold increase in phospho-c-Jun signal detected in UV-treated HEK 293 compared to untreated cell lysate. Notably higher levels of phosphorylation were also detected for phosphorylated p70 S6 kinase and phospho-ATF-2 assays in UV-treated cell lysates. The signals for untreated cell lysates were consistently low across all diluted samples. The low signals, around 60–70 MFI, in untreated cell lysates made the ratio of UV-treated to untreated as high as 76 at 12 µg of total protein concentration. Lower phosphorylation levels were detected for phospho-p53 (Ser15) and phospho-JNK (Thr183/Tyr185), but the signal differences between untreated and UV-treated cell lysates were still significant at all tested protein concentrations.
Data from this 6-plex run (one total target assay multiplexed with five phosphoprotein assays) suggests that it is practical to multiplex Bio-Plex total target assays with Bio-Plex phosphoprotein assays, provided that the targets are not the same.

Total ERK2 Assay Results Correlated With Protein Concentration
Total ERK2 assay showed consistent results in both untreated and UV-treated HEK 293 cell lysates. The signal for total ERK2, though not linear, showed a good dose-response over the range from 12 to 0.6 µg/ml. The same was true for the phosphoproteins in each of the treated samples. These results emphasize the importance of establishing the dose-response relationship between the concentration of each protein target and the corresponding signal when a specific cell lysate sample is tested. These results demonstrate that multiplexing a total assay with five phosphoprotein assays can detect differences among samples with different protein concentrations.

Fig. 1. Bio-Plex phosphoprotein and total ERK2 assays in lysates from untreated and UV-treated HEK 293 cells. Signal is plotted as relative mean fluorescent intensity (MFI) values for various protein loads. Error bars indicate SD. Light blue bars, ERK2 signal (data shown in all panels for ease of comparison); dark blue bars, phosphoprotein signal.
Introduction

Cytokines and chemokines are important soluble factors that facilitate the response of a host immune system to invading microorganisms. These soluble factors are secreted by a variety of cell types. Initially, local cells produce chemokines and pro-inflammatory cytokines that lead to the expression of adhesion molecules and recruitment of circulating leukocytes into infected tissue. The leukocytes then secrete additional chemokines and cytokines. An example of this process occurs in the eye as a result of corneal infection with herpes simplex virus type 1. Infection of the cornea with herpes simplex virus type 1 prompts a massive infiltration of neutrophils, followed by macrophages and lymphocytes. The expression of pro-inflammatory cytokines (for example, IL-1) and chemokines (for example, CCL3 and CXCL10) drives this cellular response (Biswas et al. 2004, Carr et al. 2003, Tumpey et al. 1998). Similarly to the inflammatory process in the eye, genital infection with herpes simplex virus type 2 (HSV-2) leads to the local expression and secretion of soluble mediators, including IFN-γ. These mediators are critical in controlling the viral infection (Milligan and Bernstein 1997). It is the combination of these soluble mediators that ultimately dictates the outcome of the host response, presumably by aiding the clearance of the invading pathogen.

Analysis of the host response during the infectious process requires the measurement of multiple cytokine or chemokine species. At times, the analysis is limited by the amount of available tissue, because the sample is small or the number of different parameters being measured is large. Accordingly, using a system that allows multiple cytokine and chemokine species to be measured simultaneously in a given sample enlarges the repertoire of data without severely limiting the sample for other uses.

The present study was undertaken to compare the results obtained with a conventional sandwich ELISA format to those obtained with the Bio-Plex cytokine assay. Specifically, the cytokines IL-6 and IFN-γ and the chemokine CCL5 were quantitated in three different tissues using each method.

Methods

Mice and Infection

C57BL/6 female mice aged 6–10 weeks were used. Five days before infection, the mice were given 2.0 mg of Depo-Provera subcutaneously to immunosuppress the animals, facilitating ease of infection (Kaushic et al. 2003). After 5 days, the mice were infected intravaginally with a clinical isolate of HSV-2 in 20 µl of RPMI (20,000 pfu/vagina). Seven days postinfection, the mice were euthanized and perfused with phosphate-buffered saline (PBS), pH 7.4. The vaginal tissue, spinal cord,
and inguinal lymph nodes were removed and homogenized in PBS containing 1x protease inhibitor cocktail set 1 (Calbiochem/EMD Biosciences, San Diego, CA). The homogenized tissue was centrifuged at 4°C (10,000 x g, 1 min), and the clarified supernatant was transferred to 500 ml snap-cap tubes and immediately frozen at –80°C until analysis.

**Multiplex Assay and ELISA**

A Bio-Plex mouse cytokine assay capable of simultaneously measuring IL-6, IL-10, IL-12, IFN-γ, and CCL5 was run. The same samples were then analyzed for IL-6, IFN-γ, and CCL5 content using commercially available sandwich ELISA kits (Quantikine immunoassay, R&D Systems, Minneapolis, MN).

**Results**

In response to genital HSV-2 infection, IL-6 was detected in 8/19 samples measured by the sandwich ELISA, and in 19/19 samples measured using the Bio-Plex assay (Figure 1). Furthermore, the sensitivity and range of the Bio-Plex assay were superior to the sandwich ELISA (Table 1). Linear regression analysis revealed a good correlation between the levels detected in each of the tissues by the two methods. However, the variability within each group was greater in samples measured by ELISA (ranging from 27 to 45%) compared to the Bio-Plex assay (19–31%). In contrast to IL-6, IFN-γ was detected in all tissues by both assays (Figure 1). Both assays had similar sensitivities based on detection of comparable amounts of IFN-γ in a given tissue (Table 1). However, the range of detection was again superior using the Bio-Plex assay in comparison to the ELISA method, and the variability was again greater in samples measured by ELISA (17–46%) compared to the Bio-Plex assay (12–28%). With the exception of the lymph node samples (correlation coefficient of 0.8663), both assays correlated well in the detection of IFN-γ. With respect to CCL5 levels, the sensitivity and range of detection using the Bio-Plex assay were greater than that found using the ELISA kit (Figure 1, Table 1). The correlation in the levels of CCL5 when comparing the two methods was not nearly as robust as what was found for IL-6 detection, with the exception of the spinal cord (correlation coefficient of 0.9977). Both assays had similar levels of variability in detection of CCL5, although the Bio-Plex assay was slightly better (Table 1).

**Discussion**

The ability to simultaneously measure multiple cytokines and chemokines from a small quantity of sample extends the capacity of sample analysis. The results of this study demonstrate the strength of the Bio-Plex assay for performing multiple analyses on a single sample — achieving greater range and sensitivity compared to commercially available sandwich ELISA kits. The Bio-Plex assay also reduces the potential for pipetting error, thus minimizing variability — a finding consistent with the data presented in Table 1. An assay with greater sensitivity allows detection of low levels of analyte in a sample, while a greater range minimizes the need for repeated dilutions of samples in which the target analyte is too concentrated, saving time and sample allotments. Overall, the Bio-Plex suspension array system is a valuable addition to the researcher's set of resources for efficiently measuring soluble cytokines and chemokines extracted from inflamed tissue. This unique platform saves the investigator time, consumes less sample, and produces less variable results than alternative methods.

**References**

Blewes PS et al., Mice transgenic for IL-1 receptor antagonist protein are resistant to herpetic stromal keratitis: Possible role for IL-1 in herpetic stromal keratitis pathogenesis, J Immunol 172, 3736–3744 (2004)


Milligan GN and Bernstein DI, Interferon-gamma enhances resolution of herpes simplex virus type 2 infection of the murine genital tract, Virology 229, 259–268 (1997)


**Table 1. Comparison of sensitivity, range, and variability of Bio-Plex assays and sandwich ELISA in detection of cytokine levels in HSV-2-infected tissue.***

<table>
<thead>
<tr>
<th>Assay range (pg/tissue)</th>
<th>IL-6</th>
<th>IFN-γ</th>
<th>CCL5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Plex assay</td>
<td>2–32,000**</td>
<td>2–32,000**</td>
<td>2–32,000**</td>
</tr>
<tr>
<td>ELISA</td>
<td>15–250**</td>
<td>11–600**</td>
<td>15–250**</td>
</tr>
<tr>
<td>Linearity of standard curve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-Plex assay</td>
<td>0.9036</td>
<td>0.8700</td>
<td>0.9301</td>
</tr>
<tr>
<td>ELISA</td>
<td>0.9995</td>
<td>0.9976</td>
<td>0.9880</td>
</tr>
<tr>
<td>Mean concentration (pg/tissue) detected in vagina</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-Plex assay</td>
<td>84 ± 26***</td>
<td>47 ± 12</td>
<td>1,824 ± 174†</td>
</tr>
<tr>
<td>ELISA</td>
<td>11 ± 5</td>
<td>74 ± 34</td>
<td>586 ± 117</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9683</td>
<td>0.9651</td>
<td>0.8678</td>
</tr>
<tr>
<td>Mean concentration (pg/tissue) detected in spinal cord</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-Plex assay</td>
<td>68 ± 19</td>
<td>101 ± 38</td>
<td>141 ± 54</td>
</tr>
<tr>
<td>ELISA</td>
<td>14 ± 5</td>
<td>74 ± 34</td>
<td>585 ± 117</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.8544</td>
<td>0.8966</td>
<td>0.9977</td>
</tr>
<tr>
<td>Mean concentration (pg/tissue) detected in inguinal lymph nodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-Plex assay</td>
<td>1,529 ± 116**</td>
<td>361 ± 3</td>
<td>361 ± 3</td>
</tr>
<tr>
<td>ELISA</td>
<td>26 ± 3</td>
<td>29 ± 5</td>
<td>361 ± 3</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9111</td>
<td>0.8663</td>
<td>0.8482</td>
</tr>
</tbody>
</table>

* Vaginal tissue (n = 5/assay), spinal cord (n = 5/assay), and inguinal lymph nodes (n = 9/assay).

** Range typically found in performing ELISA and Bio-Plex assays.

*** p < 0.05.
† p < 0.01 comparing the Bio-Plex to the ELISA assay as determined by Bonferonni t-test.
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**Highly Efficient Transfection of Mouse ES Cells With TransFectin™ Lipid Reagent**

Silke De-Zolt, Corinna Strolz, and Joachim Altschmied, Molecular Hematology, University Hospital Frankfurt, Theodor Stern Kai 7 D-6590, Frankfurt, Germany

**Introduction**

Embryonic stem (ES) cells have become invaluable tools in the generation of animal models with single gene mutations, which are used in research areas as diverse as developmental biology, genomics, or cancer research. In addition, ES cells provide a powerful resource to study early differentiation processes because of their unlimited capacity for self-renewal and their potential to differentiate into a number of different cell types and tissues.

The introduction of genetic material into these cells is not only required for the generation of mutated alleles, but also can be used to study the function of specific proteins or RNAs in the differentiation programs mentioned above. This can be achieved by overexpression of the respective genes or the reduction of endogenous transcript levels by expressing small hairpin RNAs inducing RNA interference (Kunath et al. 2003).

There are several established methods for the introduction of DNA into ES cells. Electroporation (Doetschman et al. 1988), on one hand, is fast, but requires large numbers of cells and DNA and subjects the cells to extremely harsh experimental conditions, leading to low survival rates. Retroviral gene transfer (Blesch 2004), on the other hand, provides very effective means for the introduction of foreign genetic material. However, it is time consuming, as it involves the production of infectious retroviral particles in a packaging cell line before the final target cell can be infected. In addition, there is a size limit to the retroviral genome that can be packaged into a virion, sometimes providing a practical obstacle when large DNA segments have to be transferred into a recipient cell. Also, the use of retroviruses invariably leads to stable integration of the provirus in the host genome, which, due to the insertional mutagenesis event associated with it, is not always desired, for example, in situations where only transient expression of a gene is necessary.

These days, a number of differently formulated transfection reagents are available that have been used successfully in many cultured mammalian cell lines. Most of them are simple to use and exhibit low toxicity. Here we compare three such reagents (based on different classes of chemical compounds) with respect to their efficiency in the transfection of a mouse ES cell line using an enhanced Green Fluorescent Protein (EGFP) expression vector to estimate the number of transfected cells.

**Methods**

**Transfection**

For transfection of E14 mouse ES cells (Hooper et al. 1987) in the absence of a feeder layer, standard 24-well tissue culture plates were coated with gelatin by covering the culture surface for 5 min with a 0.1% (w/v) aqueous gelatin solution. Directly after aspiration of the gelatin, 10^5 cells per well were seeded in regular growth medium (DMEM, 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 µM nonessential amino acids, 1,500 U/ml leukemia inhibitory factor, 10^{-6} M β-mercaptoethanol) and allowed to attach to the surface overnight. The next day, the cells were transfected with one of three commercially available transfection reagents: Bio-Rad's TransFectin transfection reagent, another supplier's linear polyethyleneimine, which acts as a “proton sponge” (reagent A), or a third supplier's nonliposomal blend of lipids (reagent B). For transfection with TransFectin, 50 µl growth medium without serum was mixed with 0.8 µg plasmid DNA and another 50 µl of the same medium with 2 µl TransFectin. Both mixtures were combined and incubated for 20 min at room temperature. During this time, the cells were re-fed with 500 µl complete growth medium containing serum. Afterwards, the transfection solution was added dropwise to the wells. The TransFectin-containing medium was removed 4 hr later, and the cells were cultivated with fresh growth medium until analysis. Transfections with other reagents were performed according to the manufacturers' specifications.

**Analysis of Transfection Efficiency**

A derivative of the reporter plasmid pEGFP-C (Clontech) was used to quantitate transfection efficiencies. In this derivative, the EGFp coding sequence was placed under transcriptional control of the human elongation factor 1α (EF-1α) gene promoter. Control transfections were performed with plasmid pcDNA3.1 (Invitrogen).
ES cells were harvested by trypsinization 2 days after transfection, collected by centrifugation for 5 min at 180 x g, washed once with 2 ml PBS, resuspended in 500 µl 2% (v/v) paraformaldehyde in PBS, and analyzed using a FACScan system (Beckton-Dickinson).

Results
Whereas at most 3% of the cells could be transfected with the two other commercially available reagents, the use of TransFectin resulted in about 20% of cells expressing the plasmid-coded EGFP gene, indicating a roughly 7-fold higher transfection efficiency (Figure 1). This efficiency was obtained with only a 4 hr TransFectin treatment, after which no obvious damage of the cells was visible by microscopic observation.

Conclusions
Especially in situations where only short-term transient expression of foreign genes in mouse ES cells is required, such as when recombinases like Cre or Flp are used for genomic engineering purposes and selective pressure cannot be applied, it is mandatory to transfect a large proportion of the cells. Efficient transfection is also advantageous when the derivation of stably transfected cells is desired. We have shown that a fairly short treatment of mouse E14 ES cells with TransFectin is sufficient to obtain a large proportion of transfected cells. It is possible that even higher transfection rates could be achieved by longer incubation with the reagent. However, it seems advisable to keep treatments of ES cells, which are generally used for differentiation studies or generation of whole animals, as short as possible to avoid any adverse effects on pluripotency and differentiation capacity.

References

Fig. 1. Transfection efficiencies of E14 mouse ES cells obtained using three commercially available transfection reagents. Shown is the percentage of EGFP-positive cells (mean ± SEM of three independent transfections) 2 days after transfection with a reporter plasmid, pEGFP-C (blue bars), or a control plasmid, pcDNA3.1 (red bars). Control expression was <0.1% for all three reagents.
Purification of Recombinant Adeno-Associated Virus Type 2 Using UNOsphere™ Media

Julia Zhen, Bio-Rad Laboratories, Inc., Hercules CA 94547 USA

Introduction
Viruses are uniquely capable of efficiently delivering nucleic acid to specific cell types while avoiding immunosurveillance by an infected host. This ability makes viruses attractive gene-delivery vehicles, or vectors, for gene therapy. Several types of viruses, including retrovirus, herpes simplex virus, adenovirus, and adeno-associated virus, have been modified for use in gene therapy applications.

Recombinant adeno-associated virus (rAAV) vectors are derived from adeno-associated virus (AAV), a defective, nonpathogenic human parvovirus that inserts its genome in a site-specific manner into a broad range of cells. The rAAV vectors in use today, in contrast to other viral gene-delivery systems, show long-term gene expression in vivo without causing cytotoxic reactions by the host immune system. These vectors have been used successfully in a large range of cell types and tissues, and preclinical studies have demonstrated the utility of rAAVs for long-lasting genetic modification and gene therapy.

Recent advances in rAAV production have increased vector titers (Zolotukhin et al. 1999), eliminated contamination by helper adenovirus (Clark et al. 1999, Drittanti et al. 2001), and added more options for regulatable gene expression and targeted cell types (Haberman et al. 1998). Unfortunately, conventional methods for rAAV purification have not kept pace with these developments. Traditional techniques such as ultracentrifugation with CsCl gradients produce preparations of variable quality, result in significant loss of particle infectivity, and are difficult to scale up to produce the quantities of vector required for clinical testing.

More recent purification strategies involve the combination of nonionic iodixanol gradients followed by ion exchange or heparin affinity chromatography (Zolotukhin et al. 1999), or a combination of one or two steps of heparin affinity chromatography (Auricchio et al. 2001, Gao et al. 2000a), ion exchange chromatography (Blouin et al. 2004, Gao et al. 2000b, Kaludov et al. 2002), or chromatography on ceramic hydroxyapatite and DEAE-Sepharose (O’Riordan et al. 2000). These methods also present drawbacks. Heparin-based approaches are expensive and require special FDA approval. Several of the other approaches show poor recoveries and require either an ultracentrifugation step, which makes them difficult to scale up, or a pretreatment of cellular extracts, which may introduce reagents that are difficult to remove downstream. In short, current published rAAV purification methods are not capable of providing the vector in sufficient quantity and purity for large-scale clinical human applications.

This study explores the use of Bio-Rad’s process-scale chromatographic media for the isolation of a highly purified recombinant adeno-associated virus type 2 (rAAV2) vector. Eight resins were screened for their ability to bind rAAV2, and a method was developed that applies a combination of ion exchange supports for the purification of rAAV2 from crude cell lysates. The method uses UNOsphere Q support for initial cleanup of lysates and UNOsphere S support for subsequent purification of rAAV2. This method, scalable and very suitable for large-scale production, provides 68% recovery of infectious virus.

Methods
Sample Preparation
Recombinant AAV type 2 virus containing Green Fluorescent Protein (rAAV2-GFP) and human HEK 293 cells containing rAAV2-GFP were used. Cell lysates were prepared by subjecting rAAV2-GFP-containing cells to three freeze/thaw cycles with an ethanol/dry ice bath. The lysate was clarified by centrifugation twice at 9,000 rpm. Aliquots of the clarified supernatant were stored at –70°C.

Batch Screening
Eight Bio-Rad chromatography resins were screened for their ability to bind rAAV2-GFP: UNOsphere Q, UNOsphere S, Macro-Prep® High Q, Macro-Prep High S, Macro-Prep DEAE, Macro-Prep CM, and CHT™ ceramic hydroxyapatite, Types I and II.

Each resin was equilibrated in 80 mM sodium phosphate under nine different pH conditions (pH 5.0–9.0 in 0.5 pH unit increments). After the buffer was drained, 1 x 10^{11} rAAV2-GFP particles and 2 ml of the same buffer were loaded into each test tube. The mixture of resin and rAAV2-GFP particles was agitated for 2 hr and the supernatant was collected. Each tube containing the resin was then washed with 4 ml of buffer; the supernatant was collected. Finally, virus was eluted with 2 hr of agitation in 0.5 ml of the same buffer plus 2 M NaCl.
Eluted samples were analyzed by SDS-PAGE for the presence of viral capsid proteins. SDS-PAGE was performed with Criterion™ precast gels using the Criterion cell and the PowerPac™ 3000 power supply. Gels were stained overnight with Ruby Red stain and imaged with the Molecular Imager FX™ system.

**Purification Process Method Development**

The BioLogic DuoFlow™ workstation was used for the chromatographic separations described here. A series of Bio-Scale™ MT2 columns (7 x 52 mm) was packed with 1.7 ml UNOsphere S or UNOsphere Q support in 20 mM sodium phosphate buffer, pH 7.5. Sample was loaded onto the columns in the same buffer using a flow rate of 150 cm/hr. The sample was eluted in a 5 column volume (CV) step gradient.

To determine the optimal salt concentration for elution of rAAV2-GFP from the UNOsphere S support, concentrations of NaCl from 50 mM to 1 M at 50–100 mM increments were tested using a 5 CV step gradient. The percent recovery of rAAV2-GFP was determined by extrapolation from SDS-PAGE densitometry of a series of known concentrations of the VP3 capsid protein.

The initial screen revealed that UNOsphere Q support did not bind rAAV2-GFP, so we investigated using this support to remove impurities from the rAAV2-GFP, which eluted in the flow-through. Clarified cell lysate was applied to an 11 x 50 mm Bio-Scale UNOsphere Q column. The column was subsequently washed with a 5 CV step gradient (0–1 M NaCl) at a flow rate of 1 ml/min. The flow-through from the Bio-Scale UNOsphere Q column was applied to a 7 x 52 mm Bio-Scale column containing UNOsphere S. The rAAV2-GFP particles were eluted in a 5 CV step gradient (0–0.35 M NaCl) at a flow rate of 1 ml/min.

**Results and Discussion**

Of the eight Bio-Rad process-scale supports examined, only UNOsphere S support showed strong binding of rAAV2-GFP. Figure 1 shows an SDS-PAGE analysis of eluates from UNOsphere S and demonstrates the significant binding of rAAV2-GFP particles on this support at a range of pH values.

The optimal salt concentration for elution of rAAV2-GFP from the UNOsphere S column was 350 mM (Figure 2), and its recovery under these conditions was about 68% (Figures 3 and 4).

Though rAAV2-GFP did not bind to UNOsphere Q support, passing the crude 293 cell lysate through the UNOsphere Q column did remove many impurities. The rAAV2-GFP in the flow-through fractions (Figure 5A) were then collected and loaded onto an UNOsphere S column. The elution profile of rAAV2-GFP from the column is shown in Figure 5B, and SDS-PAGE analysis of the 350 mM NaCl elution fraction is shown in Figure 6.

The rAAV2-GFP vector retained biological activity following chromatography on the UNOsphere S column. Human 293 cells treated with control rAAV2-GFP vector, with the

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**Fig. 1. SDS-PAGE analysis of rAAV2-GFP elution from UNOsphere S support at different pH values.** Left lane, rAAV2-GFP control (C); remaining lanes, elutions at pH 5.0–9.0. Arrow indicates position of the VP3 capsid protein.

**Fig. 2. rAAV2-GFP elution from UNOsphere S support at different concentrations of NaCl.** The NaCl concentration was increased incrementally. Representative fractions are shown after SDS-PAGE. MW, protein standard; C, rAAV2-GFP control; L, sample load; R, resquilibrium. Arrow indicates position of the VP3 capsid protein.

**Fig. 3. rAAV2-GFP elution from an UNOsphere S column in 0.35 M NaCl.** Arrow indicates rAAV2-GFP peak. Blue trace, A280; red trace, conductivity (the 5 CV elution was followed by a wash step at 1 M NaCl).

**Fig. 4. SDS-PAGE analysis of rAAV2-GFP recovery from UNOsphere S support.** MW, protein standard; L, sample load; E, 350 mM NaCl elution. Equal proportions of the total sample were loaded for comparison to the dilution series of the rAAV2-GFP control. Arrow indicates position of the VP3 capsid protein.
UNOsphere S flow-through, or with the 350 mM NaCl UNOsphere S eluate were examined by fluorescence microscopy to assess GFP fluorescence (Figure 7). By comparing the numbers of fluorescent cells in the control and the experimental cell populations, we confirmed that rAAV2-GFP recovery on the UNOsphere S column was 68%.

Conclusions
We describe a method suitable for large-scale purification of rAAV2 vector using two ion exchange chromatography supports in series: UNOsphere Q is used in flow-through mode to bind contaminants in the cell lysate. UNOsphere S is used for purification of rAAV2-GFP particles. Though UNOsphere Q does not bind rAAV2-GFP, it does bind genomic DNA and other impurities in human cell lysates. It removed substantial amounts of these impurities when used as a first-step purification. The flow-through from the UNOsphere Q column was then applied to an UNOsphere S column, used to capture the rAAV2-GFP virus particles from the partially purified lysate. The virus particles elute from the support with 350 mM NaCl, 20 mM phosphate buffer, pH 7.5. These supports and buffers are well suited to process-scale applications. This procedure provides 68% recovery of clean, biologically active rAAV2-GFP particles from human cell lysates.

Acknowledgement
Special thanks to Dr Xiao Xiao, University of Pittsburgh, for providing the rAAV2-GFP material and his numerous contributions to this project.

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Blouin V et al., Improving rAAV production and purification: towards the definition of a scalable process, J Gene Med Suppl 1, S223–S228 (2004)
Clark KR et al., Highly purified recombinant adeno-associated virus vectors are biologically active and free of detectable helper and wild-type viruses, Hum Gene Ther 10, 1031–1039 (1999)
Printing Uniform Spots With the VersArray ChipWriter™ Systems

Many factors can affect the uniformity of spots when printing arrays with robotic arrayers, including either the VersArray ChipWriter™ Pro or VersArray ChipWriter™ Compact system. This lack of uniformity may affect hybridization, and ultimately yield poor-quality data. Among the critical factors are the concentration of the sample to be spotted, the substrate (slide) surface, and the printing parameters, as well as the printing pins, printhead, environmental conditions, and immobilization of the sample on the substrate. This tip concentrates on these latter four factors.

When printing DNA or oligonucleotide arrays, it is relatively easy to evaluate the quality of spots through a prehybridization technique. This involves staining the array with a fluorescent dye that binds to the DNA, or with a fluorescently labeled random nanomer. The slide can then be scanned in a VersArray ChipReader™ system and assessed for missing spots, spot morphology (that is, the “roundness” of each spot), and the relative amount of DNA immobilized on the slide.

To determine whether printing problems are caused by a pin or the printhead, the subarrays in the image should be examined for localized imperfections. Large differences among subarrays can indicate a damaged pin, a dirty pin, or a dirty printhead. Pins should be cleaned following each print run and examined under a microscope for defects prior to a new run. A dirty printhead that causes the pins to stick can be remedied by thorough cleaning with 70% ethanol using a small brush, such as those used as interdental devices.

Poor spot morphology that is not confined to one subarray can result from printing at nonoptimal temperature or humidity. High temperature and humidity typically yield larger spots than lower temperature and humidity. If the spot dries too fast and the DNA is not allowed to diffuse uniformly, a donut- or odd-shaped spot may result. Adjusting the temperature and humidity can help alleviate these printing problems. Typically 20°C and 55% relative humidity are recommended for DNA arrays.

If the array signal intensity seems abnormally low, the amount of DNA immobilized may be the problem. If this is the case, the UV crosslinker should be assessed by identifying the proper UV settings for optimal binding. The images in the figure below were obtained from an experiment designed to test the crosslinker. The top image revealed the unit was functioning properly, while the bottom image was due to a faulty unit.

Further Reading

Automated Electrophoresis
• Experion™ automated electrophoresis system brochure (bulletin 3140)
• Experion Pro260 analysis kit product information sheet (bulletin 3169)
• Experion RNA HighSens and RNA StdSens analysis kits product information sheet (bulletin 3170)
• Experion software product information sheet (bulletin 3171)
• Experion automated electrophoresis system video tour (bulletin 3184)
• Experion automated electrophoresis system folder (bulletin 3174A, includes bulletins 3140, 3169, 3170, 3171, and 3184)

Nucleic Acid Amplification
• Amplification resource CD (all tech notes; bulletin 3167)
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• Comparison of Cibacron Blue affinity-based formats for depletion of albumin from samples prior to two-dimensional gel electrophoresis (bulletin 3168, PDF only)
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