

# TWO-DIMENSIONAL GEL ELECTROPHORESIS

## 2-D in a Day

- **Meaningful 2-D results in <24 hours**
- **Increased number of visible protein spots with narrow overlapping pH range ReadyStrip IPG strips**
- **Increased resolving power, with the mini gel advantages of speed and easy handling**

## Significant Protein Resolution in a Fraction of the Time

### Introduction

Two-dimensional electrophoresis is a cornerstone of proteomics research: It is used to identify and analyze differences in protein expression in proteome studies, and to monitor global protein expression. However, 2-D electrophoresis has a reputation for being a time-consuming, technical application. With new products from Bio-Rad, the time and effort required to do 2-D electrophoresis is reduced so that you can now do 2-D in a day.

The following protocol was used to generate the results shown in the figure below. A complete user manual is included with each of the products.

**Tuesday, 3 PM** Rehydrate 11 cm ReadyStrip™ IPG strip in protein sample and buffer for 12 hr.

**Wednesday, 3 AM** Rehydration is complete. Isoelectric focusing program starts automatically and continues for 7 hr.

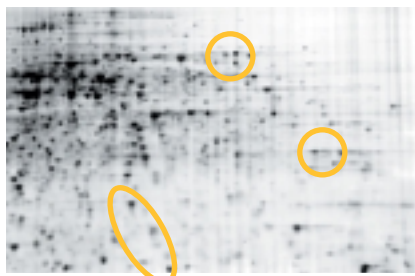
**Wednesday, 10 AM** IPG strip is removed from focusing tray and equilibrated in buffer in preparation for the second-dimension run.

**Wednesday, 10:30 AM** IPG strip is loaded onto Criterion™ gel and run is started.

**Wednesday, 11:30 AM** Run is complete. Gel is stained with either Bio-Safe™ Coomassie stain or SYPRO Ruby protein gel stain.

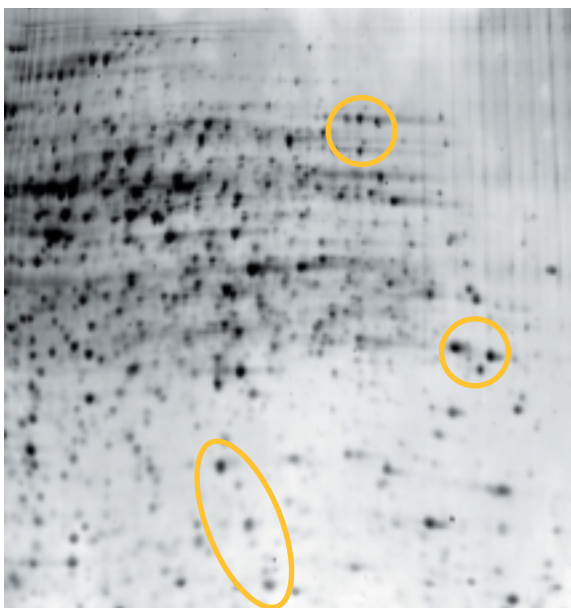
**Wednesday, 2:30 PM** 2-D gel is ready for imaging and spot excision.

A



2-D gels generated using the Criterion format (A) and the PROTEAN® II format (B). The Criterion format offers a shorter processing time, with ease of spot detection approaching that of a large gel. Increase total resolution further by using narrow pH range ReadyStrip IPG strips. Three regions of orientation landmarks are circled on each gel.

B

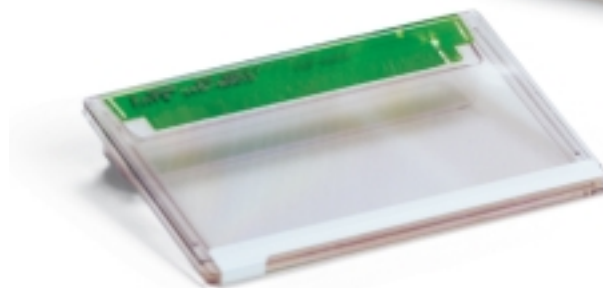


**BIO-RAD**

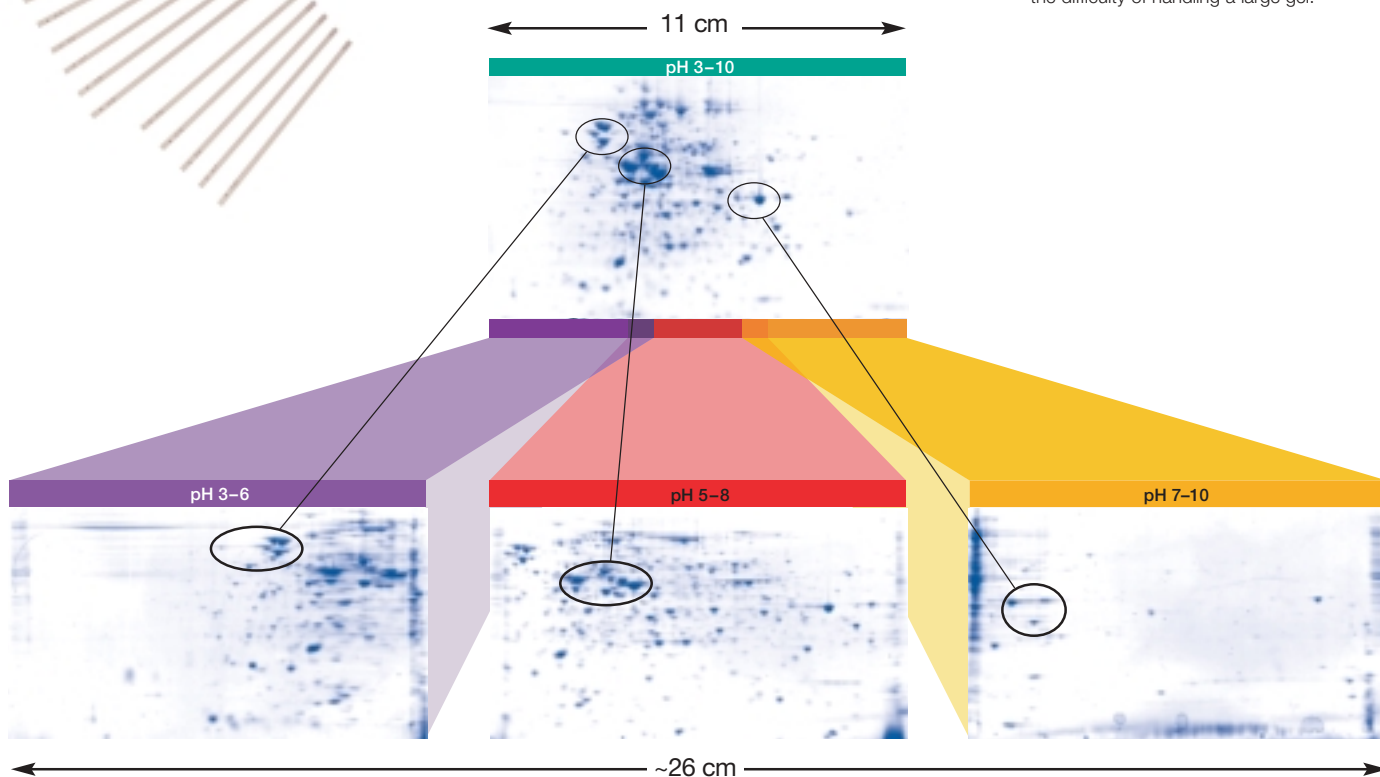
# Increase

resolving power in the first dimension with narrow overlapping pH range

## ReadyStrip™ IPG strips



The Criterion system is the new standard in precast gel systems; it stretches the traditional mini format, providing 60% more resolving area in the first dimension, and 24% more resolving area in the second dimension. Get more separation power without the difficulty of handling a large gel.



Increase in resolving power in the first dimension with overlapping pH range IPG strips. *E. coli* lysate (40 µg per gel) was run on 11 cm narrow overlapping pH range ReadyStrip IPG strips and focused to 20,000 V-hr. The strips were then transferred to 8–16% Tris-HCl Criterion precast gels for the second-dimension run. The gels were stained with colloidal Coomassie Blue. More proteins are detected on the 3 overlapping gels than on the single 3–10 pH range gel. Note the improved resolution of proteins in the circled areas.

## A sample **2-D in a Day** protocol:

### Sample Preparation

Washed *E. coli* cells are suspended in chilled 20 mM Tris-HCl, pH 8.0. The cells are sonicated twice at 1 min intervals; the sonicator is set to power level 2. The suspension is centrifuged for 20 min at 10,000 x g, 4°C. The protein concentration of the supernatant is approximately 3–5 mg/ml.

The supernatant is diluted into rehydration buffer (8 M urea, 2% CHAPS, 10 mM DTT, 0.2% Bio-Lyte® 3/10 ampholyte) to a final protein concentration of 0.3–0.5 mg/ml. A 200 µl aliquot is pipetted into the focusing tray.

An 11 cm ReadyStrip IPG strip, pH 4–7, is wetted in the solution containing sample and buffer, and mineral oil is pipetted over the strip to prevent precipitation of the urea.

### First-Dimension Isoelectric Focusing

A rehydration sequence is programmed on the PROTEAN IEF cell. The temperature of the Peltier platform is set to 20°C, and the strip is allowed to rehydrate for about 12 hr under passive conditions (no voltage applied). The PROTEAN IEF cell is programmed to begin the focusing step automatically after 12 hr. The strip is focused for a total of 40,000–50,000 V-hr. For this experiment,\* the 11 cm ReadyStrip IPG strip was run at 6,000 V for a total of 40,000 V-hr. (A Hold step can be programmed to follow focusing, so that 500 V is applied to the strip while waiting to begin second-dimension SDS-PAGE.)

To prepare the ReadyStrip IPG strip for the second dimension, the strip must be removed from the focusing tray and equilibrated in 5 ml of buffer containing 50 mM Tris-HCl, pH 8.8, 6 M urea, 2% SDS, 30% glycerol, and 1% DTT. A second equilibration in the same buffer with 1.5% iodoacetamide substituted for the DTT follows.

### Second-Dimension SDS-PAGE

The ReadyStrip IPG strip is transferred onto a Criterion gel (8–16% gradient) and overlaid with 0.5% agarose in Laemmli buffer. The gel is run for 60 min at 200 V. Following electrophoresis, the gel is removed from the cassette and placed in 100 ml of SYPRO Ruby protein gel stain for at least 3 hr.

\* A maximum of 8,000 V and 50 mA can be applied to an 11 cm ReadyStrip IPG strip.

\*\* Actual gel size is 13.3 x 8.7 cm.

### Technical Tip #1:

Samples with protein concentrations <3 mg/ml should be concentrated prior to dilution in rehydration buffer. Similarly, protein samples with salt concentration >100 mM should be desalted.

### Technical Tip #2:

Bromophenol Blue is sometimes used in the rehydration buffer to verify even distribution of sample along the strip. However, it too is an ionic species, and including it in the buffer may require an increase in the number of volt-hours required to focus a sample.

### Technical Tip #3:

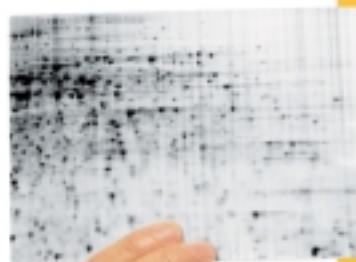
Running duplicate strips saves time during protocol optimization. One strip can be stained immediately to verify that focusing conditions are appropriate. The unstained strip can then be used for the second-dimension run.

### Technical Tip #4:

It is critical that both equilibration solutions be prepared immediately before use to ensure full effectiveness of the reducing agents.

### Technical Tip #5:

The gel can remain in SYPRO Ruby protein gel stain for up to 24 hr without risk of overstaining the gel.



Mingde Zhu, staff scientist, Bio-Rad Laboratories, with the final result of the “2-D in a Day” protocol.\*\*





The PROTEAN IEF cell and ReadyStrip IPG strips streamline handling and resolution in the first dimension.



The Criterion Dodeca™ cell is perfect for high-throughput gel analysis since it accommodates up to 12 Criterion gels per run.

## Product Selection Guide

Catalog # Description

### Sample Preparation Kits and Reagents

|          |  |
|----------|--|
| 163-2100 | ReadyPrep™ Sequential Extraction Kit, based on the method of Molloy et al. (Electrophoresis 19, 837–844, 1998) |
| 163-2105 | 2-D Starter Kit  |
| 163-2106 | ReadyPrep 2-D Starter Kit Rehydration/Sample Buffer (for approximately fifty 11 cm strips)                     |
| 163-2107 | ReadyPrep 2-D Starter Kit Equilibration Buffer I (for approximately five 11 cm strips)                         |
| 163-2108 | ReadyPrep 2-D Starter Kit Equilibration Buffer II (for approximately five 11 cm strips)                        |
| 163-2110 | <i>E. coli</i> Protein Sample, lyophilized, 2.7 mg   |
| 163-2111 | ReadyPrep Overlay Agarose, 50 ml   |
| 161-0731 | Urea, 1 kg   |
| 161-0611 | DTT, 5 g   |
| 163-2109 | Iodoacetamide, 30 g  |
| 161-0460 | CHAPS, 1 g   |

### PROTEAN IEF System

|          |   |
|----------|---|
| 165-4000 | PROTEAN IEF System, complete, includes basic unit, 17, 11, and 7 cm focusing trays with lids, 1 pack each of 17, 11, and 7 cm rehydration/equilibration trays with lids, 2 pair forceps, electrode wicks, mineral oil, cleaning brushes |
|----------|---|

### ReadyStrip IPG Strips (Also Available in 7, 17, 18, and 24 cm)

|          |   |
|----------|---|
| 163-2014 | ReadyStrip IPG Strips, 11 cm, pH range 3–10, 12           |
| 163-2016 | ReadyStrip IPG Strips, 11 cm, pH range 3–10 nonlinear, 12 |
| 163-2017 | ReadyStrip IPG Strips, 11 cm, pH range 3–6, 12            |
| 163-2015 | ReadyStrip IPG Strips, 11 cm, pH range 4–7, 12            |
| 163-2018 | ReadyStrip IPG Strips, 11 cm, pH range 5–8, 12            |
| 163-2019 | ReadyStrip IPG Strips, 11 cm, pH range 7–10, 12           |
| 163-2024 | ReadyStrip IPG Strips, 11 cm, pH range 3.9–5.1, 12        |
| 163-2025 | ReadyStrip IPG Strips, 11 cm, pH range 4.7–5.9, 12        |
| 163-2026 | ReadyStrip IPG Strips, 11 cm, pH range 5.5–6.7, 12        |
| 163-2027 | ReadyStrip IPG Strips, 11 cm, pH range 6.3–8.3, 12        |

### Bio-Lyte IEF Buffers

|          |                                      |
|----------|--------------------------------------|
| 163-2094 | 100x Bio-Lyte 3/10 Ampholyte, 1 ml   |
| 163-2093 | 100x ReadyStrip 7–10 Buffer, 1 ml    |
| 163-2098 | 100x ReadyStrip 3.9–5.1 Buffer, 1 ml |
| 163-2097 | 100x ReadyStrip 4.7–5.9 Buffer, 1 ml |
| 163-2096 | 100x ReadyStrip 5.5–6.7 Buffer, 1 ml |
| 163-2095 | 100x ReadyStrip 6.3–8.3 Buffer, 1 ml |

Catalog # Description

### Criterion Systems for SDS-PAGE

|          |   |
|----------|---|
| 165-6001 | Criterion Cell, includes tank, lid with power cables, 3 sample loading guides |
| 165-4130 | Criterion Dodeca Cell, includes tank and lid with power cables, instructions  |
| 164-5050 | PowerPac Basic™ Power Supply, 100–120/220–240 V                               |
| 165-5052 | PowerPac 200 Power Supply, 100/120 V  |
| 165-5053 | PowerPac 200 Power Supply, 220/240 V  |

### Criterion Precast Gels, Tris-HCl, IPG+1 Comb, 11 cm IPG Strip

|          |   |
|----------|---|
| 345-0101 | 10% resolving gel, 4% stacking gel      |
| 345-0102 | 12.5% resolving gel, 4% stacking gel    |
| 345-0103 | 4–15% resolving gel, no stacking gel    |
| 345-0104 | 4–20% resolving gel, no stacking gel    |
| 345-0105 | 8–16% resolving gel, 4% stacking gel    |
| 345-0106 | 10.5–14% resolving gel, 4% stacking gel |
| 345-0107 | 10–20% resolving gel, 4% stacking gel   |

### Electrophoresis Reagents

|          |                                |
|----------|--------------------------------|
| 161-0732 | 10x Tris/Glycine/SDS, 1 L      |
| 161-0772 | 10x Tris/Glycine/SDS, 5 L cube |

### Criterion XT Bis-Tris Precast Gels, IPG+1 Comb, 11 cm IPG Strip

|          |                     |
|----------|---------------------|
| 345-0115 | 10% resolving gel   |
| 345-0121 | 12% resolving gel   |
| 345-0127 | 4–12% resolving gel |

### Criterion XT Tris-Acetate Precast Gels, IPG+1 Comb, 11 cm IPG Strip

|          |                    |
|----------|--------------------|
| 345-0133 | 7% resolving gel   |
| 345-0139 | 3–8% resolving gel |

### Criterion XT Buffers and Reagents

|          |  |
|----------|--|
| 161-0788 | XT MOPS Running Buffer, 20x, 500 ml  |
| 161-0789 | XT MES Running Buffer, 20x, 500 ml   |
| 161-0790 | XT Tricine Running Buffer, 20x, 500 ml   |
| 161-0791 | XT Sample Buffer, 4x, 10 ml  |
| 161-0792 | XT Reducing Agent, 1 ml  |
| 161-0793 | XT MOPS Buffer Kit, includes 500 ml 20x XT MOPS running buffer, 10 ml 4x sample buffer, 1 ml XT reducing agent       |
| 161-0796 | XT MES Buffer Kit, includes 500 ml 20x XT MES running buffer, 10 ml 4x sample buffer, 1 ml XT reducing agent         |
| 161-0797 | XT Tricine Buffer Kit, includes 500 ml 20x XT Tricine running buffer, 10 ml 4x sample buffer, 1 ml XT reducing agent |

### Protein Stains

|          |   |
|----------|---|
| 170-3125 | SYPRO Ruby Protein Gel Stain, 1 L (stains 10 Criterion gels)    |
| 161-0786 | Bio-Safe Coomassie Stain, 1 L bottle (stains 10 Criterion gels) |
| 161-0787 | Bio-Safe Coomassie Stain, 5 L cube (stains 50 Criterion gels)   |

Highlighted catalog numbers are products used to obtain the “2-D in a Day” results on a Criterion precast gel (Tris-HCl, IPG comb, 8–16%, catalog #345-0041).

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