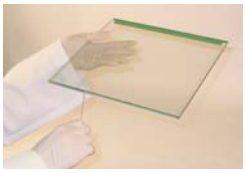
PROTEAN® Plus Precast Gels
Quick Guide

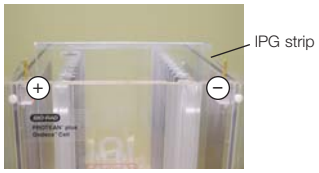
Expression Proteomics // Tools for Protein Separation and Analysis www.expressionproteomics.com

1 Pre-Run Preparation



- Prepare the running buffer (see the formulation on other side)
- Remove a gel from its pouch and peel the tape off of the bottom edge of the gel cassette. Tear along the plane of the cassette towards the right; **note:** do not pull up or down on the tape, but only from left to right
- Remove the comb and rinse the well with running buffer. Dry the well with thin filter paper or by holding the cassette upside down and letting excess buffer drip out

- Place the gel on an AnyGel™ stand
- Load the IPG strip (and protein standards, if desired; **note:** liquid standards are not recommended)
- Overlay with agarose (refer to other side for details)



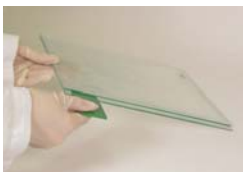
- Once the agarose has solidified, rotate the gel 90° and insert it into the PROTEAN Plus Dodeca™ cell as shown above
- Check the following:
  - That the top of the gel (the end holding the IPG strip) is positioned next to the cathode (negative/black electrode card)
  - That the level of buffer in the tank is midway through the upper spacer; make certain the entire gel area is submerged in buffer

2 Electrophoresis

Connect the PROTEAN Plus Dodeca cell to a PowerPac™ HC or PowerPac™ Universal power supply. Turn on the power supply, refrigerated cooler, and buffer recirculation pump. Set the power supply to 150 V and run it for 18–20 hr. When electrophoresis is complete, turn off the power supply, buffer recirculation pump, and refrigerated circulator. **Disconnect the leads from the power supply prior to removing the lid.** Remove gels from the buffer tank using both hands.

3 Gel Removal

- Place the gel cassette on the benchtop, with the short plate facing towards you
- Remove a spacer from either side of cassette (or both sides) by pulling the tabs at the top of the cassette. Be sure to pull along the plane of the cassette towards the bottom; **note:** do not pull the tab towards or away from you



- Insert a gel releaser (catalog #165-3320) between the glass plates at the top right corner. Pull the gel releaser up, lifting the short plate
- Continue lifting the short plate until it is separated completely; in most cases, the gel will stick to the longer plate

- Use a water bottle to squirt water along the side and bottom edges to loosen the gel from the glass
- To remove the gel, slide a wetted gel clip (catalog #165-3414) under the gel. To ensure a secure grip on the gel, hold the gel clip with both hands and make sure that at least 1–1.5 cm of the gel is inside the clip. Lift the gel slowly from the glass plate, transfer it to a staining tray or other container, and stain using the appropriate protocol

BIO-RAD

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PROTEAN® Plus Precast Gels
Instructions

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Introduction

PROTEAN Plus precast gels are large format polyacrylamide 2-D gels for use in the PROTEAN Plus Dodeca™ cell. They provide optimum high-throughput second-dimension separation, excellent reproducibility, and bar coding for ease of tracking.

I. Running Buffer Formulation

1x Tris/glycine/SDS: 25 mM Tris base (MW 121.1), 192 mM glycine (MW 75.07), 0.1% SDS, pH 8.3

II. Loading IPG Strips

Use these instructions for any commercially available IPG strips, including Bio-Rad ReadyStrip™ IPG strips and Amersham Biosciences dry strips. For strip preparation prior to loading onto the gel, refer to the ReadyStrip IPG strip instruction manual, bulletin 4006166.

1. Position the PROTEAN Plus precast gel upright, leaning it backwards slightly. Bio-Rad's AnyGel™ stands are designed to simplify IPG strip loading onto precast gels. A test tube rack may also be used.
2. Prepare 50–100 ml of running buffer in a graduated cylinder. Using forceps, dip the IPG strip into the buffer-filled cylinder to rinse off the equilibration solution.
3. After rinsing, place the strip lengthwise across the PROTEAN Plus gel, with the plastic backing of the strip against the longer back plate. Use a thin spatula to push the strip down between the two plates so that it rests against the gel. Be careful to push against the plastic backing of the strip instead of the gel strip itself to avoid damaging the gel.

III. Agarose Overlay

1. Prepare 0.75% agarose in SDS-PAGE running buffer. Premixed PROTEAN Plus overlay agarose (catalog #163-2092) is also available.
2. Pour the agarose over the IPG strip so the overlay completely covers the strip.
3. Bubbles may form under or around the strip when pouring the overlay. Remove these bubbles if possible, as they can adversely affect resolution. To remove bubbles, use a spatula and tap the top of the IPG strip against the plastic backing.
4. Allow the agarose overlay to solidify completely (~2 min) prior to inserting the gel into the cell.

Specifications	
Gel format	24 cm IPG strip*
Gel dimensions (W x L)	25.6 x 23.0 cm
Gel thickness	1.0 mm
Cassette dimensions (W x L)	27.0 x 25.0 cm
Cassette material	Recyclable glass
Comb material	Polycarbonate
Buffer volume	23 L for 12 gels
Gel shelf life	Tris-HCl gels: 12 weeks from date of manufacture
Storage conditions	Store flat at 4°C; do not freeze

* Includes space for loading molecular weight standards

IV. Cell Assembly

For complete details of cell assembly, including manifold tubing requirements, refer to the PROTEAN Plus Dodeca cell instruction manual, bulletin 4006203, section 3.

V. Bar Coding

PROTEAN Plus precast gels are labeled with a bar code and a human-readable, unique alphanumeric string for ease of tracking and convenience. Bar codes can be read with any standard bar code reader* (Code 128 Subset B) and entered into any database.

VI. Troubleshooting

For 2-D troubleshooting information and help, visit us on the Web at **www.expressionproteomics.com**. There you will find the 2-D Doctor, an interactive self-help troubleshooting guide, as well as other supporting resources.

Ordering Information

Catalog #	Description
PROTEAN Plus Precast Gel	
161-1511	PROTEAN Plus Tris-HCl Gel, 12%, 24 cm IPG well
Premixed Buffers	
161-0732	10x Tris/Glycine/SDS, 1 L
161-0772	10x Tris/Glycine/SDS, 5 L cube
161-0734	10x Tris/Glycine, 1 L
161-0771	10x Tris/Glycine, 5 L cube
Accessories	
163-2092	PROTEAN Plus Overlay Agarose, 125 ml
165-4131	AnyGel Stand, single-row, holds 1 PROTEAN Plus gel, 2 Criterion™ gels, or 3 mini gels
165-5131	AnyGel Stand, 6-row, holds 6 PROTEAN Plus gels, 12 Criterion gels, or 18 mini gels
165-3320	Mini-PROTEAN® 3 Gel Releasers
165-3414	Gel Clip
165-3400	Dodeca Stainer, large, 100–240 V

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* For more information on readers, contact PSC Inc., www.psc.com