PROTEAN® II xi Cell IPG Conversion Kit Setup Guide

This kit allows 18.5 cm wide x 20 cm long gels to be run on Bio-Rad's PROTEAN II xi Cell thus accommodating commercially available immobilized pH gradient gel strips for 2-D second dimension protein separations. It contains:

- Wide sealing gaskets for the upper buffer chamber (2)
- 20 cm glass plates (2 sets)
- 20 cm notched clamps (2 sets)
- Foam gaskets for the PROTEAN II xi Casting Stand (2)
- Alignment card
- Narrow spacers (4) *
- Prep Combs (2)*

* 1 mm or 2 mm thick depending upon which kit you ordered.

Casting Gels

Gels are cast the same way as with the standard PROTEAN II xi Cell (refer to your user manual for details). For best results, replace the solid rubber gaskets on the PROTEAN II xi Casting Stand with the foam rubber gaskets included with this kit. They provide a more reliable seal when using the narrow spacers. When assembling the gel "sandwich", use the alignment card to push the spacers to the extreme outer edges of the glass plates to ensure maximum gel width. Since the card is narrower than the gel cavity, you will need to slide it side-to-side to properly align the spacers.

After pouring the monomer solution, insert the comb into the gel. Using combs ensures gel-to-gel dimensional repeatability. **This is especially true if using the PROTEAN II xi Multi-Gel Casting Chamber**. Overlaying gels with t-amyl alcohol can be done, but unless it is applied simultaneously to all gels, the gel heights will not be the same because the weight of the overlay applied to the first gel (and each subsequent gel) will displace the solutions in the other gels.

The combs provided in this kit have two sides—one with a reference well for standards and one without. The side without the standard lane allows for commercially available 18 cm IPG strips to be used without excess trimming. The side with the end reference well allows standards to be run along side your sample. However, 18 cm IPG's will need to be trimmed more to fit into the smaller prep well. Deciding which comb side to use depends upon what you know about your sample. When running without the reference well, an internal standard can still be included by adding a known protein to your sample. Providing a marker between gels helps to monitor the reproducibility of the 2-D patterns and makes post run gel comparisons easier. To determine which standard marker to use, run a 1-D SDS gel of your unknown sample.

Assembling the Upper Buffer Chamber

Replace the standard U-shaped gaskets on the central cooling core with wider U-shaped sealing gaskets provided with this kit. (Refer to section 5.1 in the PROTEAN II xi Cell manual.)

Once the gels have polymerized, remove the gel sandwich assemblies from the casting stand, remove combs, and mount the gel sandwich assemblies onto the central cooling core to create the upper buffer chamber.

If running one gel, a buffer dam can be mounted on the opposite side of the core. If you do not have a buffer dam, clamp an inner and outer glass plate together without spacers and mount them on the central cooling core. **Note that either U-shaped gasket size will work with the buffer dam or glass plate set**.

Loading the IPG strip onto the Slab Gel

Equilibrate the IPG strip in second dimension equilibration solution as you normally would before placing them onto the second dimension vertical gel. Using a pair of forceps, manually slide the IPG strip onto the slab gel **such that one edge of the IPG strip abuts against the top of the slab gel.** Secure the IPG in place by overlaying it with molten agarose containing 1% electrode buffer (refer to Section 14.1, solution C, in the PROTEAN II xi user manual for the recipe). It is important that no bubbles be trapped between the two gels.

Running the 2nd Dimension Gel

Electrophorese the gels until the bromophenol blue marker dye runs to the bottom of the gel. Upon completion of the run, remove and fix the gels as soon as possible as described in section 9 of the PROTEAN II xi Cell user manual.

Ordering Information

Catalog Number	Product Description
165-3183	PROTEAN II xi Cell IPG Conversion Kit, 1 mm Gel*
165-3184	PROTEAN II xi Cell IPG Conversion Kit, 2 mm Gel*
165-1834	PROTEAN II xi Basic Unit with casting stand**
165-1835	IPG Clamp Set (1R and 1L)
165-1836	IPG Spacers, 1 mm, 4
165-1837	IPG Spacers, 2 mm, 4
165-1838	IPG 2-D Prep Comb, 1 mm, 1
165-1839	IPG 2-D Prep Comb, 2 mm, 1
165-3182	IPG Sealing Gasket, 2
165-1823	Inner Glass Plate, 20 cm x 20 cm, 2
165-1824	Outer Glass Plate, 22.3 cm x 20 cm, 2
165-3181	IPG Spacers, 1.5 mm, 4
165-3186	PROTEAN II xi Cell IPG Conversion Kit, 1.5 mm Gel*
165-3187	IPG 2-D Prep Comb, 1.5 mm, 1

* Kit includes 2 sets of IPG clamps, 2 sets of glass plates, 4 IPG spacers, 2 IPG 2-D/prep combs, 2 IPG gaskets, one alignment card, and two casting stand gaskets

** The PROTEAN II xi Basic Unit does not include spacers, combs, gaskets, or plates. Combine it with an IPG Conversion kit for a complete system.

See Bio-Rad catalog for standard PROTEAN II xi Cell & Multi-Gel Cell accessories.

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