2-D Electrophoresis for Proteomics — Second Generation Separation Methods

Instruction Manual

Bulletin 6222

IPG Equilibration for the Second Dimension

To solubilize focused proteins and to allow SDS binding in preparation for the second dimension, it is necessary to equilibrate focused IPG strips in SDS-containing buffers. This step is analogous to boiling samples in SDS buffer prior to 1-D SDS-PAGE. See page 14 for further discussion of equilibration. Rehydration/equilibration trays sized for each size strip can be used for equilibration.

Table 1. Equilibration base buffer.*

Reagents	Amount (Final Concentration)
Urea	36 g urea (6 M)
20% SDS	10 ml (2%)
1.5 M Tris/HCl, pH 8.8 gel buffer	3.3 ml (0.05 M)
50% Glycerol	40 ml (20%)
Water	Adjust to 100 ml

* This buffer may be frozen in aliquots. Lyophilized equilibration base buffer can be ordered as ReadyPrep™ equilibration buffer II, which reconstitutes to 20 ml.

Equilibration protocol:

Place one strip, gel side up, in each channel and fill the channels successively with the equilibration buffers derived from the base buffer (Table 1). First incubate with gentle agitation in DTT equilibration buffer 1 for 10 min, then decant. Refill the channels with iodoacetamide equilibration buffer 2 and incubate again for 10 min. This method requires 2.5 ml of each solution per strip for 7 cm strips, 4 ml for 11 cm strips, and 6 ml for 17 cm strips. After equilibration, remove the IPG strip and embed it onto the prepared second-dimension gel as described in the following section.

DTT Equilibration Buffer 1

This buffer reduces sulfhydryl groups. To prepare it, add DTT to equilibration base buffer (Table 1) to 2% (200 mg/10 ml) immediately prior to use.

Iodoacetamide Equilibration Buffer 2

This buffer alkylates sulfhydryl groups. While the strip is incubating in equilibration buffer 1, add dry iodoacetamide to equilibration base buffer (Table 1) to 2.5% (250 mg/10 ml).

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Placement and Agarose Embedding of IPG Strips

Position the second-dimension gel cassette so that it is leaning slightly backwards (approximately 30° from vertical). Place the IPG strip onto the long plate with the plastic backing against the plate. Slide the strip, face down, between the plates using a spatula to push against the plastic backing. Be careful not to damage the gel with the spatula.

Make sure the IPG strip is positioned directly on top of the second-dimension gel. To secure the strip in place, overlay it with 0.5–1.0% molten agarose prepared in SDS-PAGE running buffer (a small amount of Bromophenol Blue can be added to the agarose overlay in order to track the ion front during the run). Use warm molten agarose; hot agarose may accelerate decomposition of the urea in the equilibration buffer. Bubbles may form under or behind the strip when adding the agarose overlay. These bubbles may disturb protein migration and must be removed. Immediately after overlaying, use the spatula to dislodge bubbles by tapping the plastic backing on top of the strip. Stand the gel upright and allow the agarose to set prior to loading the gel into the electrophoresis cell (see Figure 1).

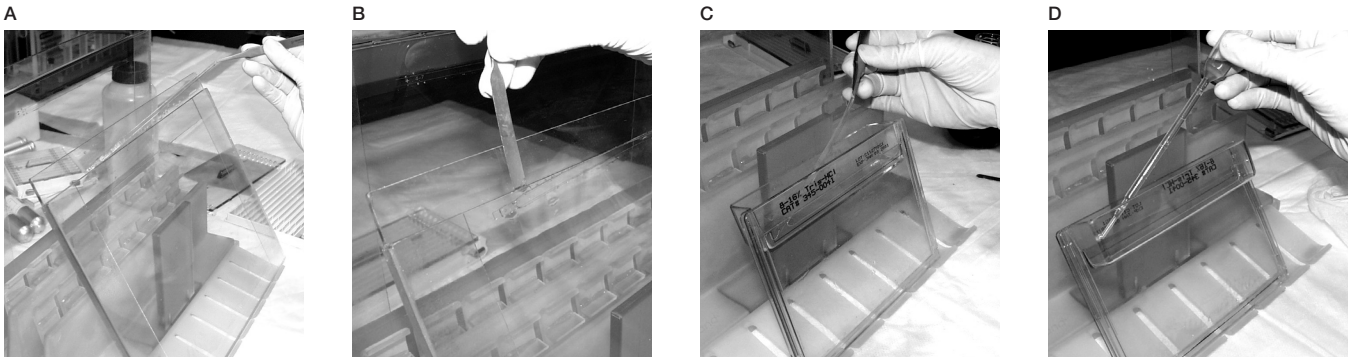

Fig. 1. A and B. In **A**, a 17 cm ReadyStrip™ IPG strip (held in forceps) is applied to the top of a PROTEAN XL Ready Gel® precast gel. The gel is held at an angle with the short plate forward. In **B**, the strip is pushed into direct contact with the top of the gel using a spatula. In **C**, an 11 cm ReadyStrip IPG strip is applied to the top of a Criterion™ precast gel held at an angle. The strip is aligned so the plastic backing is against the back plate and the IPG strip is touching the top of the gel. Molten agarose may be added before the strip is placed in the well and the strip positioned within the liquid agarose, or the agarose may be added after the strip is in position (**D**). The gel is moved to an upright position while the agarose is setting.

This is an excerpt from Bio-Rad's comprehensive manual, 2-D Electrophoresis for Proteomics (Bulletin 2651).

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