ProteoGel™ IPG Equilibration Buffer

ProductInformation

Product Code **I 7281**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description
Two-dimensional electrophoresis separates proteins by their isoelectric points (pI) in the first dimension and then by their molecular weights in the second dimension. Separation in the first dimension is achieved by focusing the solubilized proteins in a gel containing an immobilized pH gradient (e.g. ProteoGel™ IPG Strips).

Following the first dimension, the IPG strip is equilibrated in buffer to denature the proteins with SDS, buffer the strip to a pH appropriate for electrophoresis, and maintain protein solubilization. The equilibrated IPG strip is then placed in an SDS-PAGE gel for separation of the proteins by molecular weight in the second dimension.

ProteoGel™ IPG Equilibration Buffer is a convenient product for the equilibration of IPG strips prior to SDS-PAGE electrophoresis. This product can be used with all SDS-PAGE gel systems. The ProteoGel IPG Equilibration Buffer does not contain any reducing or alkylating agents.

Reconstitution of the powdered product with 35 ml of water results in 50 ml of solution containing 0.05 M tris acetate, 2% (w/v) SDS, 6 M urea, and 0.0067% (w/v) bromophenol blue, pH 7.0.

Products Required But Not Provided
Ultrapure Water (18 megohm or equivalent)
(Product Code W 4502)
Rocker (Product Code Z36,774-5)
Plastic tubes or trays for equilibration

Precautions and Disclaimer
This item is for laboratory use only, not for drug, household, or other uses. Consult the MSDS for information regarding hazards and safe handling practices.

Preparation Instructions
Add 35 ml of ultrapure water to the contents of the bottle. Mix until all solids are completely dissolved. The solution will become cold and gentle heating in a water bath may aid solubilization. To prevent the formation of cyanates from urea, do not heat above 30 °C. The final volume of the solution is 50 ml.

Note: Some of the material may stick to the side of the bottle. If this occurs, briefly heating to room temperature in a water bath will help to dissolve the material. This does not affect the performance of the ProteoGel IPG Equilibration Buffer.

ProteoGel IPG Equilibration Buffer is compatible with all types of IPG strips. Table 1 shows the minimum volume of equilibration buffer suggested for various strip sizes.

Table 1.
Equilibration Guidelines

Strip Length	Volume/ Strip	Number of Strips per Bottle (50 ml)
7 cm	3 ml	16
11 cm	5 ml	10
18 cm	8 ml	6

Storage/Stability
The powdered product can be stored at room temperature for up to two years prior to reconstitution. After reconstitution, store the equilibration buffer in working aliquots at –20 °C to prevent cyanate formation.

Procedure

Sample preparation
Samples can be prepared to load onto the IPG strips using standard methods or using one of the protein extraction kits offered by Sigma-Aldrich (see Related Products).

Rehydration and focusing of the IPG strips can be performed using standard methods.

IPG strip equilibration
Choose one of the following equilibration methods:

For samples that have been previously reduced and alkylated.

1. Place the focused IPG strip in a plastic equilibration tray or plastic tube with the plastic IPG strip backing against the container surface.
2. Pipette the appropriate volume of equilibration buffer over the strip according to Table 1.
3. Place the tray or tube(s) on a rocker such that the strips are fully covered by the equilibration solution and equilibrate the IPG strips at room temperature for 20 to 60 minutes.
   Note: Longer equilibration time may result in loss of low molecular weight proteins from the strip.
4. Take the IPG strip out of the tray or tube and blot the backing of the IPG strip on a dry paper towel to drain the excess equilibration buffer.
5. The IPG strip is ready for SDS-PAGE electrophoresis.

For reduction and alkylation during equilibration

1. Place the focused IPG strip in a plastic equilibration tray or plastic tube with the plastic IPG strip backing against the container surface.
2. Add the reducing agent (Table 2) to the appropriate volume of ProteoGel IPG Equilibration Buffer (Table 1). Addition of a reducing agent ensures that the proteins remain reduced during the second dimension electrophoresis.

Table 2.
Recommended Reducing Agent Concentration

Reducing Agent	Product Code	Final Concentration
Dithiothreitol (DTT)	D 5545	30 mM (4.5 mg/ml)
Tributyl phosphine (TBP)	T 7567	5 mM (0.04 mg/ml)
Tris(carboxyethyl) phosphine (TCEP)	C 4706	5 mM (1.5 mg/ml)

3. Pipette the solution prepared in step 2 over the IPG strip.
4. Place the tray or tube(s) on a rocker such that the strips are fully covered by the equilibration solution and equilibrate the IPG strips at room temperature for 15 to 30 minutes.
5. Decant the equilibration buffer. For samples to be reduced only, skip the alkylation steps and proceed to step 9.
6. Add the alkylating reagent, iodoacetamide (IAA) (Product Code A 3221) to a fresh aliquot of the appropriate volume of equilibration buffer (Table 1). Use a 3 to 6 fold molar excess over the concentration of reducing reagent used in step 2 (e.g. 15 to 30 mM IAA for 5 mM TBP or TCEP or 90 to 180 mM IAA for 30 mM DTT).
7. Pipette the equilibration buffer prepared in step 6 over the IPG strip.
8. Place the tray or tube(s) on a rocker such that the strips are moving freely in solution and equilibrate the IPG strips at room temperature for 15 to 30 minutes.
9. Take the IPG strip out of the tray or tube and blot the backing of the IPG strip on a dry paper towel to drain the excess equilibration buffer.
10. The IPG strip is ready for SDS-PAGE electrophoresis.

Second Dimension SDS-PAGE
IPG strips can be run on the second dimension using standard methods or by the following method:
Place the equilibrated IPG strip in the well of the 2-D gel using a forceps and/or a spatula. The IPG strip should make complete contact with the top of the gel. Because the IPG strip is in contact with the gel, no agarose overlay is required.

Related Products	Product Code
ProteoPrep™ Kits Total Extraction Sample Membrane Protein Extraction Universal Extraction	 PROT-TOT PROT-MEM PROT-TWO
ProteoGel™ Tris-Tricine-SDS Electrode Buffer	T 2821
ProteoGel™ Tris Acetate Sample Buffer 5X Concentrate	T 5196

References

(1) Gorg, A., et al., The current state of two-dimensional electrophoresis with immobilized pH gradients. Electrophoresis, **9**, 531-546 (1988).
(2) Hames, B.D., Gel Electrophoresis of Proteins: A Practical Approach. 3rd Ed. (IRL Press, Oxford, England, 1998).

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