3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

ProteoGel™ IPG Strips

Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Two-dimensional electrophoresis separates proteins in the first dimension by their isoelectric point (pI) and by molecular weight in the second dimension. Isoelectric point separation is achieved by electrophoresis (focusing) of solubilized proteins in a gel containing an immobilized pH gradient (ProteoGel™ IPG Strips). Following the first dimension separation, the ProteoGel IPG Strip is equilibrated with ProteoGel™ IPG Equilibration Buffer (Product No. I 7281) to denature the proteins with a detergent (SDS) and urea. The ProteoGel IPG Strip is then placed in the well of a SDS-PAGE 2D gel and electrophoresed to separate the proteins by molecular weight.

ProteoGel IPG Strips are produced using acrylamido buffers to create stable, immobilized pH gradients in a polyacrylamide matrix. The polyacrylamide matrix is dried onto a plastic backing to increase the shelf-life of the IPG strips and to allow the strip to be rehydrated with the protein sample.

ProteoGel IPG Strips are available with a wide range pH gradient (pH 3-10) or 5 different narrow range pH gradients (3-5, 4-7, 5-8, 6-11, and 8-11) to optimize the separation of the proteins. The strips are available in three different lengths (7 cm, 11 cm, and 18 cm). The strips are conveniently labeled with a "+" on the acidic (anode) end of the strip for orientation in the focusing unit.

Table 1.
ProteoGel IPG Strip Product Codes

	pH Range					
Length	3-10	3-5	4-7	5-8	6-11	8-11
7 cm	I 2531	I 3031	I 2906	I 3156	I 7406	I 3281
11 cm	I 3406	I 3656	I 3531	I 3781	I 7531	I 3906
18 cm	I 4031	I 4281	I 4156	I 4406	I 7656	I 4531

Products Required But Not Provided

Ultrapure Water (18 megohm or equivalent)
Rehydration tray
Forceps (Product No. F 3767)
Mineral oil (Product No. M 3516)
IPG strip focusing apparatus
ProteoGel IPG Equilibration Buffer (Product No. I 7281)
SDS-PAGE apparatus
Gel Protein Stain
- Coomassie Brilliant Blue G-250 stain
  (EZBlue™ Gel Staining Reagent, Product No. G 1041) **or**
- Silver Stain
  (ProteoSilver™ Silver Stain Kit, Product Code PROT-SIL1) or (ProteoSilver™ Plus, Product Code PROT-SIL2 for samples prepared for MALDI-MS analysis.)

DuraSeal laboratory stretch film (Product No. D 3172) or Parafilm (Product No. P 7793)

Precautions and Disclaimer

These products are for laboratory use only, not for drug, household, or other uses. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

The ProteoGel IPG strips are stable at –20 °C for at least 1 year in an unopened package.

Procedure

1. Prepare the protein sample in an appropriate solubilization/extraction solution. Sigma offers three different ProteoPrep™ kits (PROT-TOT, PROT-TWO, and PROT-MEM) for 2D electrophoresis sample preparation. Each kit contains solubilization/extraction solutions, a tributylphosphine solution for reduction of the sample, and iodoacetamide for protein alkylation.

2. Dilute an aliquot of the prepared sample to the desired concentration. Pipette the appropriate amount of sample (see Table 2) along the edge of the rehydration tray.

Table 2.
ProteoGel IPG strip rehydration volumes

IPG strip length	Sample volume
7 cm	125 µl
11 cm	200 µl
18 cm	320 µl

3. Remove the protective plastic from the ProteoGel IPG strip gel surface and place the strip, gel side down, on the sample such that the entire gel is in contact with sample.
   Note: When the gel side is down, the writing on the strip will appear in the correct orientation. Wrap the rehydration tray with laboratory stretch film to prevent evaporation. Allow the strips to rehydrate at room temperature for 5 hours or until essentially all the sample has been absorbed into the gel. Lower temperatures may cause the urea to precipitate. Less than 3 µl of the sample should remain in the tray after rehydration.
   Note: Water-saturated blotting paper may be added to an empty lane of the rehydration tray to reduce evaporation or overlay mineral oil (Product No. M 3516) on the strips.
4. Assemble the strip into the IPG strip focusing apparatus following the manufacturer's instructions. The acidic end (+) of the ProteoGel IPG strip should be at the anode (red/+). Ensure that the gel on the IPG strip has made contact with the electrode or a moist electrode wick. Overlay mineral oil on the strips to minimize evaporation during the focusing.
5. See Table 3 for the recommended electrophoresis protocols for focusing of the ProteoGel IPG strips. The recommended temperature is 20 °C. Lower temperatures may cause the urea to precipitate. Increasing the total volt hours may improve the focusing. The maximum current allowed per strip is 50 µA, otherwise damage to the strip from overheating may occur.

Table 3.
ProteoGel IPG Strip Focusing Conditions

Step	Voltage	Time	Volt Hours
7 cm strip			
Conditioning	250 V	1 hour	
Ramp	250 – 6000 V	2 hours	
Focusing	6000 V		60,000
11 cm strip			
Conditioning	250 V	1 hour	
Ramp	250 – 6000 V	2 hours	
Focusing	6000 V		80,000
18 cm strip			
Conditioning	250 V	1 hour	
Ramp	250 – 6,000 V	2 hours	
Focusing	6,000 V		100,000

6. If necessary, the focused ProteoGel IPG strips may be wrapped with laboratory stretch film and stored below –20 °C for up to 1 week, prior to running the second dimension gel.
7. After focusing, equilibrate the focused IPG Strip with ProteoGel IPG Equilibration Buffer for 20 to 30 minutes at room temperature.
8. Fill the well of a SDS-PAGE 2D gel with electrode buffer and place the ProteoGel IPG Strip into the well, with forceps, so that the side of the strip makes complete contact with the top of the polyacrylamide gel. Avoid air bubbles between the strip and the top of the gel.
   Note: An agarose overlay is not necessary.
9. Assemble the SDS-PAGE 2D gel into the electrophoresis unit and electrophorese the gel until the blue dye front is within 1 cm of the bottom of the gel.
10. Stain the SDS-PAGE gel using Coomassie Brilliant Blue (EZBlue Gel Staining Reagent, Product No. G 1041) or silver stain (ProteoSilver Silver Stain Kit, Product Code PROT-SIL1) to visualize the proteins. Proteosilver Plus (Product Code PROT-SIL2) is recommended for samples prepared for MALDI-MS analysis.

Related Products	Product Code
ProteoPrep Kits Total Extraction Sample Membrane Protein Extraction Universal Extraction	 PROT-TOT PROT-MEM PROT-TWO
Cellular and Organelle Membrane Solubilizing Reagent	C 0356
Chaotropic Membrane Extraction Reagent 2	C 0606
Dithiothreitol (DTT)	D 5545
Iodoacetamide (IAA)	A 3221
Tributylphosphine (TBP)	T 7567
ProteoGel IPG Equilibration Buffer	I 7281
ProteoGel Tris-Tricine-SDS Electrode Buffer	T 2821
Hi/Lo Profile Rocker	Z36,774-5

References

1. Gorg, Angelika, Two-Dimensional Electrophoresis of Proteins Using Immobilized pH Gradients. A Laboratory Manual. Technical University of Munich (Munich, Germany: 1998).

Technology developed in partnership with Proteome Systems™.

MDS/MAM 3/02