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IsoGel® Agarose IEF Plates, pH 6-10.5

Introduction

IsoGel® Agarose IEF Plates, pH 6-10.5 are prepared by custom blending IsoGel® Agarose with ampholytes. They form a less restrictive matrix than polyacrylamide and are ideal for rapid focusing of high molecular weight proteins (>200 kDa). They are particularly well suited for the separation and analysis of antibodies. IsoGel® Agarose is cast on GelBond® Film to provide dimensional stability and ease of handling throughout processing. Plate dimensions are 100 mm x 125 mm.

Storage

Store plates at 4°C

Materials Required

- Sample Applicator Mask
- Electrode Wicks
- Blotting Paper
  (The above three items are included in the IsoGel® IEF Plate Accessory Pack, Catalog No. 56014,
  100 wicks and 100 sheets blotting paper are
  Catalog No. 56010)
- Horizontal Isoelectric Focusing Chamber. The Multiphor® II Electrophoresis Unit from Amersham Pharmacia BioTech is ideal for use with IsoGel® Agarose IEF Plates.
- Constant Power Supply
- Refrigerated Circulator Bath
- Forced Air Oven set at 50°C to 55°C
- pI Marker Proteins
- Micropipettor and Pipette tips

Electrolyte Solutions Required

- Anolyte: 0.05 M Glacial Acetic Acid **OR** 0.1 M Glutamic acid (Sigma G-5513)
  Add 0.147 g to 10 ml dH_2O, do not adjust pH
- Catholyte: Cathode Fluid 10, Cresent Chemical Catalog #42986.03

Preparing Protein Samples

For protein samples containing salts, dialyze the protein sample against one of the following:

- Deionized water
- 1% glycine
- 0.05 M to 0.1 M ammonium bicarbonate.

For samples that are hydrophobic or poorly soluble, add either nonionic or zwitterionic detergent to the sample at a final concentration of 0.05% to 1.0%

Nonionic detergents

- Triton® X-100
- Nonidet® (NP-40)
- Tween® 80

Zwitterionic detergents

- CHAPS
- Zwittergent® 3-14

Circulation Bath

Set the temperature on the circulator bath so that the platen temperature reaches 10°C-15°C. Chilling must occur enough in advance to ensure platen temperature is 10°C.

Isoelectric Focusing Procedure

NOTE: These instructions are meant for gels run in the **Portrait orientation** (10 cm wide and 12.5 cm long). Running the gels in the Landscape orientation (12.5 cm wide and 10 cm long) may alter sample resolution.

Removing IsoGel® Plate From Cassette

NOTE: Remove gel from refrigerator and use immediately. Gels left at room temperature for extended periods may adhere to the upper cassette when the cassette is opened.

NOTE: A frosted or crystalline background on a gel when first opened may indicate the product was frozen. If this occurs contact Technical Service for assistance.

1. Cut the foil storage bag and remove the plastic cassette.
2. With the opening on the cassette facing downward, remove the cassette cover by placing a spatula tip between the two halves of the cassette and twist.
3. Remove the gel by gently pushing a finger through the opening in the cassette bottom.

Gel Placement

1. Wipe any condensation off the cooling platen. The surface of the electrophoresis platform should be between 10°C and 15°C.
2. Spread a small volume (100 µl) of deionized water onto the center of the cooling platen of the IEF chamber. This will hold the gel to the platen by capillary action.
3. Lower the gel on the wetted area.
   NOTE: Avoid trapping air between the GelBond® Film and platen. If bubbles form, use the edge of a scalpel blade or thin spatula to gently lift the edge of the gel, then lay the gel back down on the platen to remove the bubble.

4. Wipe or blot excess fluid from the area around the gel, right up to the edges of the gel.
5. Briefly blot the surface of the gel with a sheet of blotting paper.

Electrode Wick Application

1. Cut wicks to 9 cm in length. This length is ideal because it allows 0.5 cm space between the edges of the gel and each end of the wick.
2. Completely immerse a wick in the catholyte solution (Cathode Fluid 10). This is the cathodal wick.
3. Remove excess fluid from the wick by placing it briefly on blotting paper, and then gently blot the top surface of the wick with blotting paper.
4. Repeat steps 1 and 2 with another wick using the anolyte solution (0.1 M Glutamic acid). This is the anodal wick.
5. Place the cathodal wick at the (-) electrode contact of the gel, centered on the gel.
6. Place the anodal wick at the (+) electrode contact of the gel, centered on the gel.
7. Verify that the wicks are parallel and in even, smooth contact with the gel surface.
8. Lay a glass plate on top of both wicks for 10 to 15 seconds to compress the wicks evenly on the gel surface.

Sample Application

1. Place the sample applicator mask across the gel parallel with the wicks. Positioning of sample application may be adjusted depending on the pI of the proteins. We recommend loading samples 3 cm from anode wick.
2. Pipette 2 µl to 5 µl of sample solution into the applicator mask slots.
3. Place electrode assemblies onto the wicks, and align the electrodes so they are parallel with the gel.
   Tip: To minimize excess pressure on the IsoGel® Agarose IEF Plate, cut four, 4 cm long wicks. Place the wicks on the four corners of the electrophoresis platform, approximately 1.5 cm in from the short sides of the platform, lying parallel to the short sides of the platform. Position the wicks so that they cushion the electrodes, relieving pressure on the gel.
4. Set the power supply at 1 W constant power and apply power for 10 minutes to prefocus the samples.
5. Turn off the power and remove the sample applicator mask. The applicator mask can be reused. Rinse with deionized water prior to storing.
6. Gently remove any precipitated sample from the gel surface with blotting paper.

Focusing Samples

1. Apply power to the gel as follows (initial settings):

Power Limit	Voltage Limit	Focusing Time
25 W	1000 V	60 to 90 minutes

2. Monitor the separation by:
   - Observing the separation of visible marker proteins
   - A drop in current to a low, steady state (1 mA-5 mA)
3. When focusing is complete, turn off power, discard the wicks and place the gel in fixative.

Fixing, Staining and Destaining

Background

Either Coomassie® Brilliant Blue or Crowle's Double Stain can be used to stain proteins in IsoGel® Agarose IEF Plates. Coomassie® Brilliant Blue Stain will give the highest sensitivity. Crowle's Double Stain will give the clearest background, and slightly lower sensitivity. Individual proteins may stain more intensely with one or the other stain.

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Preparation of Fixative for either Coomassie® or Crowle's Stain

Fixative Solution
180 ml Methanol
30.0 g Trichloroacetic acid
18.0 g Sulfosalicylic acid (e.g., Sigma catalog S-2130)
Fill to 500 ml with deionized water

Preparation of Coomassie® Staining Solutions

Coomassie® Blue Stain
1.0 g Coomassie® Brilliant Blue R-250
250 ml Ethanol
90 ml Glacial acetic acid
Fill to 1 L with deionized water

Coomassie® Destaining Solution
250 ml Ethanol
90 ml Glacial acetic acid
Fill to 1 L with deionized water

Crowle's Double Stain
2.5 g Crocein scarlet (C.I. 26905)
150.0 mg Coomassie® Brilliant Blue R-250
50 ml Glacial acetic acid
30.0 g Trichloroacetic acid
Fill to 1 L with deionized water

Crowle's Destaining
Tap water

Gel Fixation

1. Place the gel in fixative for 20 to 30 minutes. (Rubbermaid® Servin Saver® Rectangle #3865 or Rubbermaid® Servin Saver® 2 or 5 cup square containers work well.)
2. Remove the gel from the fixative by grasping onto the edge of the gel film with forceps.
3. Place the gel on a paper towel, gel side up.

Gel Drying

1. Wet a single sheet of Whatman® 3MM Blotting Paper with deionized water.
2. Place the wetted Whatman® 3MM Paper on the gel surface and smooth to remove any air bubbles.
3. Overlay the paper with six layers of absorbent paper toweling.
4. Place a glass plate on the paper towels. Weight it down with a 1 kg-2 kg weight.
5. Leave in place for 20 minutes to overnight.
6. Remove the weight, glass plate and paper towels. Re-wet the blotting paper to allow easy removal from the gel surface, and remove it.
7. Wash the gel for 5 minutes with deionized water.
8. Dry the gel in a 50°C to 55°C forced hot air oven for a minimum of 30 minutes, overnight is fine. Gels may also be left to dry overnight at room temperature.

Gel Staining Crowle's Stain

1. Float the gel in the stain, gel side down, for 15 to 30 minutes. Floating gel side down in Crowle's Stain prevents particles in the stain solution from clinging to the gel.
2. Remove the gel from the Crowle's Stain, and gently rinse with running tap water until excess stain is removed. Gels

may be rinsed by placing in a 1500 ml beaker under a gentle stream of tap water.

Gel Staining Coomassie® Stain

1. Stain gel 15 minutes in Coomassie® Stain with gentle agitation.
2. Rinse gel in destaining solution 15-30 minutes with gentle agitation.

Gel Drying And Preservation

Place in a 50°C to 55°C hot air oven for 15 minutes.

OR:

Dry at room temperature overnight.

NOTE: Dried gels can be stored indefinitely.

For Laboratory Use.

For more information contact Technical Service at (800) 521-0390 or visit our website at www.Lonza.com