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IsoGel® Agarose

Agarose for Isoelectric Focusing

Introduction

IsoGel® Agarose for isoelectric focusing (IEF) is highly purified and specially manufactured to yield unmeasurably low electroendosmosis (EEO). An IsoGel® Agarose gel is easy to prepare and produces a gel with high clarity and less restrictive matrix than polyacrylamide, thereby allowing rapid focusing of higher molecular weight proteins (≤200 kD). Focused proteins can be transferred from the porous agarose matrix or detected by immunological techniques. It is also possible to transfer proteins from these agarose gels into a second dimension polyacrylamide or agarose sieving gel.

The porous nature of IsoGel® Agarose means you can readily fix, stain and destain your gel in a fraction of the time needed for polyacrylamide IEF. IsoGel® Agarose gels have very low background stain and proteins are easily visualized by conventional protein stains.

IsoGel® Agarose IEF gels are sufficiently rigid to allow casting of vertical tube gels in addition to vertically molded and horizontally open cast thin gels. Gels cast on GelBond® Support Film remain adhered to the film from the time of casting through isofocusing, staining and destaining. Agarose gels cast on GelBond® Film dry down quickly and easily to transparent, permanent records suitable for scanning or mounting in a laboratory notebook. A modified Silver Stain procedure has been developed for use with agarose gels cast on GelBond® Film.

A technique for direct tissue isoelectric focusing (DTIF) of soluble proteins has been developed for IsoGel® Agarose whereby tissue samples can be applied directly to the gel without the need for extraction.

IsoGel® Agarose IEF gels are compatible with immunofixation, crossed immunoelectrofocusing and preparative IEF techniques.

Apparatus Required

- Isoelectric focusing chamber
- Constant power supply
- Refrigerated circulator bath
- Forced air oven
- Sample applicator mask
- Blotting paper
- Electrode wicks
- GelBond® Film, 110 mm x 125 mm
- 1-cc tuberculin syringe
- 20-cc syringe with a large gauge needle

Reagents Required

- IsoGel® Agarose
- Ampholytes
- d-sorbitol
- pI markers
- Electrolyte solutions

Electrolytes

When selecting anolytes and catholytes for any particular pH gradient, it is important to closely bracket the ends of the pH range of the ampholytes. Avoid creating pH discontinuities between the ends of the ampholyte ranges and the bracketing electrolytes.

Anolyte	**Concentration**	**pH (25ºC)***
Phosphoric acid	1 M	1.0
Sulfuric acid	0.2 M	1.6
Acetic acid	0.5 M	2.6
L-glutamic acid	40 mM	3.2
Indole acetic acid	3 mM	3.8
L-tyrosine	4 mM	4.5
Catholyte	**Concentration**	**pH (25ºC)***
Threonine	50 mM	5.8
Glycine	50 mM	6.15
Hepes	0.4 M	7.3
L-Histidine (free-base)**	40 mM	7.35
Bicine	0.1 M	8.0
Sodium hydroxide	1 M	13.0

*pH is dependent on temperature. 25ºC pH values are provided for selection of electrolytes. Under running conditions the pH will be slightly higher.
**Do not substitute Histidine HCL for free-base.

IsoGel® Plate Preparation

The following example details the preparation of a 1% IsoGel® Agarose gel, 0.8 mm thick, cast on a 110 mm x 125 mm sheet of GelBond® Film. The total solution volume to be prepared is 10 ml. Alternatively, a larger IsoGel® Agarose solution may be prepared (without ampholytes) and stored refrigerated in tightly sealed aliquots for later remelting, addition of ampholytes, and casting into single gels.

Gel Casting Assembly
Assemble the materials required for gel casting

- Two thick glass plates (size 110 mm x 125 mm)
- One sheet of GelBond® Film, 110 mm x 125 mm
- A plastic 0.8 mm thick U-frame spacer or 3 single spacers, 0.8 mm thick
- Six stationery binder clamps

Casting Cassette Assembly
1. Spread a few drops of distilled water or 0.1% nonionic detergent on one glass plate.
2. Lay a sheet of GelBond® Film, cut slightly smaller than the glass plate, on to the plate with the hydrophilic side up (water droplets spread on the hydrophilic side but bead up on the hydrophobic side).
3. Cover the GelBond® Film with a sheet of blotting paper or the interleaving paper supplied with the GelBond® Film and firmly roll with a rubber roller or wipe with tissues to squeeze out any air bubbles and excess fluid from behind the GelBond® Film. Carefully wipe off any excess liquid at the edges.
4. Place the U-frame spacer on top of the GelBond® Film, or place two spacers on the GelBond® Film along either side and one across the bottom edge. Place the second glass plate over the spacer(s). Clamp the assembly with the stationery clamps using 2 clamps per side and bottom.
5. Warm the cassette in a 55ºC forced hot air oven for 15 minutes.
   NOTE: GelBond® Film may warp if the cassette is heated too long or above 75ºC.

Preparation of the Gel Solution
In both the following procedures, add room temperature distilled water and a magnetic stir bar to a beaker or flask that is 2-4 times the volume of the solution; sprinkle in the agarose powder while the solution is stirred to prevent formation of clumps.

Boiling Water Bath or Hot Plate Procedure
1. Weigh out separately 0.1 g IsoGel® Agarose and 1.0 g d-sorbitol.
2. Add 8 ml distilled water to the flask or beaker and slowly sprinkle the agarose powder into the distilled water with constant stirring. Using a spatula, break up and disperse any agarose clumps and scrape down any powder adhered to the walls of the flask.
3. Add the sorbitol to the beaker with constant stirring.
4. Record tare weight of the beaker and contents.
5. Cover the beaker with plastic wrap and pierce a small hole in it.
6. Heat while stirring to 90ºC-95ºC and boil for 10 minutes or until the agarose is dissolved.

Microwave Procedure
1. Follow steps 1 thru 4 above.
2. Remove the magnetic stir bar. Cover the flask with plastic wrap and pierce a small hole.
3. Heat in the microwave oven on MEDIUM (70%) power setting for 2 minutes.
4. Remove the container from oven and GENTLY swirl to resuspend settled powder and gel pieces. Reheat on HIGH (100%) power setting for 1-2 min., or until the solution comes to a boil.
5. Remove from microwave and GENTLY swirl to thoroughly mix the agarose solution.

For either dissolution method we recommend taring the beaker and solution before boiling. After dissolution, add sufficient warm distilled water to obtain initial weight and mix. Cool the solution to approximately 60ºC.

1. Add 0.63 ml of ampholytes 40% solution with a 1.0 cc tuberculin syringe to the cooled solution.
2. Stir the solution well to mix. Maintain the IsoGel®/sorbitol/ampholyte solution at 60ºC-65ºC until the time of casting the agarose gel. Correct for evaporation by adding warm distilled water immediately before transferring the solution to the casting assembly.
3. Flush the 20-cc syringe with boiling water to thoroughly heat it.
4. Expel all water from the barrel and needle.
5. Immediately fill the syringe with the warmed agarose solution, and inject it in a steady stream into the warmed cassette. Try to avoid introducing air bubbles into the cassette.
6. Fill the cassette to the top with agarose solution.
7. Seal the top of the cassette with parafilm or tape to prevent evaporation.
8. Allow the casting assembly to cool at room temperature.
9. Refrigerate it a 4ºC for one hour.
   NOTE: Set the refrigerated circulator bath to 10ºC-15ºC. *Do not* circulate the coolant to the IEF chamber until focusing is about to begin. This prevents condensation on the gel and platen.

Disassembly of the Casting Assemble
1. Remove the tape and all clamps.
2. With the cassette lying flat, insert a flat spatula between the glass plates and twist gently to break the seal. Carefully remove the top plate leaving the gel and the GelBond® Film attached to the back plate.
3. Remove the spacer(s) and lift the GelBond® Film from the back plate by inserting a flat spatula under the GelBond® Film and gently lift it away from the glass plate.

Preparation for Focusing
WARNING: Make certain the power supply is turned off before proceeding.

Gel Placement
1. Spread a small volume (0.2 ml-0.3 ml) of distilled water on the cooling platen of the IEF chamber.
2. Lower the gel on the wetted area. Avoid trapping air under the GelBond® Film. Wipe excess fluid from the edges of the film.
3. Blot the surface of the gel briefly with a sheet of fine-grained blotting paper.
4. If necessary, trim the edges of the gel parallel to the direction of focusing with a razor blade to ensure that the edges are even and free of cracks or small tears.

Electrode Wick Application

1. Cut two electrode wicks to the exact width of the gel or slightly shorter.
2. Using forceps to hold one end of an electrode wick, immerse it completely in the appropriate catholyte solution. Remove excess fluid by placing the wick on blotting paper. This light blotting facilitates the uptake of any surface moisture which might accumulate near the cathode during prolonged focusing.
3. Repeat this dipping procedure on a second wick using the appropriate anolyte solution for the anodal wick.
4. Place the blotted anodal wick on the gel surface at the (+) electrode contact of the gel. Place the cathodal wick on the (-) electrode contact of the gel. The wicks must lie parallel to each other on the ends of the gel, evenly touching the surface.
5. Lay a glass plate, slightly larger than the gel, on top of the gel and wicks for approximately one minute. This ensures uniformity of contact between wicks and gel and serves to smooth the wick surface in preparation for electrode contact.

Sample Application

1. Place the sample applicator mask across the gel at least 1 cm from either wick (e.g. 3 cm from cathode).
2. Before applying the sample care must be taken that salts are removed from the sample by dialysis against water, 0.05 M ammonium bicarbonate or 1% glycine.
3. Pipet the sample solutions into the slots (2µl-5 µl . maximum; 2-10 mg/ml concentration). In direct tissue isofocusing tissue samples may be placed directly onto the applicator slots. pI markers should be run on each gel to provide stainable markers on known isoelectric points and to indicate progress during the run.
4. Be sure that the electrodes and the electrical contacts are clean and that there are no breaks in the wire or ribbon.
5. Place the electrodes on the wicks (not the gel surface), aligning them so that they are parallel to the gel.
6. Set the power supply at 1 W (constant power) and apply power for 10 minutes.
7. Turn off the power and remove the sample applicator mask. The applicator mask is reusable and should be rinsed with distilled water.

8 .Gently remove any precipitated sample from the gel surface with blotting paper.

IEF Power Settings and Focusing Time

1. Set the voltage, current and power according to the appropriate running conditions listed below.
2. Start circulation of the coolant to the IEF chamber.
3. The separation progress can be monitored by observing the visible proteins in the pI markers focus and the rapid drop in current on the power supply's milliampere indicator. Focusing is attained when the visible pI markers are sharply resolved and the current has stopped decreasing significantly (less than 1 mA in 10 minutes).
4. Once focusing is complete, turn off the power, discard the wicks and place the gel in fixative.

Running Conditions

PH Range	Voltage (limiting)	Current	Power (upper limiting)	Anolyte	Catholyte	Focusing Time (Minutes)
2.5-4.5	250 V	MAX	10 W	1 M H_3PO_4	0.05 M Threonine	90
3.5-9.5	500 V	MAX	10 W	0.5 M HOAc	1 M naOH	90
3.5-9.5	1000 V	MAX	25 W	0.5 M HOAc	1 M NaOH	40
3.5-9.5	1500 V	MAX	25 W	0.5 M HOAc	1 M NaOH	30
4-6.5	500 V	MAX	10 W	2% solution of pH 2.5-4.5 Ampholyte	0.1 M Bicine	90
5-8	500 V	MAX	10 W	2% solution of pH 2.5-4.5 Ampholyte	0.1 M NaOH or 0.1 M L-Histidine (free-base)	90

*1 M H_3PO_4 can be replaced by 0.5 M acetic acid

Fixing & Staining Solutions

Fixative solution

180 ml Methanol
30 g Trichloroacetic acid
18 g Sulfosalicylic acid
Fill to 500 ml with distilled water

Either Coomassie® Blue Stain or Crowle's Stain can be used to stain IEF gels. Coomassie® Stain is used when increased sensitivity is desired, and Crowle's Double Stain produces gels with clear background and sharp resolution.

Coomassie® Stain

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

Coomassie® Destaining Solution

250 ml Ethanol
90 ml Glacial acetic acid
Fill to 1 liter with distilled water

Crowle's Double Stain

2.5 g Crocein Scarlet (C.I. 26905)
150.0 mg Coomassie® Brilliant Blue R-250
50.0 ml Glacial acetic acid
30.0 g Trichloroacetic acid
Fill to 1 liter with distilled water

Crowle's Destaining Solution

3 ml Glacial acetic acid
Fill to 1 liter with distilled water

Silver Stain

A modified Silver Stain procedure has been developed for use with agarose gels cast on GelBond® Film.

Fixative solution

180 ml Methanol
30.0 g Trichloroacetic acid
18.0 g Sulfosalicylic acid
Fill to 500 ml with distilled water

Pretreatment solution 1

2% glutaraldehyde

Pretreatment solution 2

0.01% DTT (dithiothreitol)

Silver Stain Solution A

Sodium carbonate, anhydrous
50.0 g in 1 L distilled water
(Stable for 2-3 weeks at room temp.)

Silver Stain Solution B

Combine the following solutions into 1 liter of distilled water, while mixing rapidly, **in the order given:**
2.0 g ammonium nitrate, 2.0 g silver nitrate, 10.0 g dodeca-tungstosilicic acid* and 6.7 ml 37% formaldehyde.
(Stable 1 week at room temp. Stored in the dark).

Stop Bath

1% Acetic acid
*available from Sigma Chemical Co.

Fixing, Staining and Destaining

Coomassie® Stain or Crowle's Double Stain

The following protocol applies to either stain

1. Place the gel in fixative for 10 minutes.
2. Grasp an edge of the gel film support with forceps to lift the gel from the fixative.
3. Rinse the gel surface with distilled water and drain excess solution.
4. Place the gel on a paper towel, gel side up.
5. Place a single sheet of Whatman® 3MM Blotting Paper, wetted with distilled water, on the gel surface.
6. Overlay the blotting paper with six layers of absorbent paper toweling.
7. Place a glass plate on top of the paper toweling and weight it down with a 1 kg-2 kg weight for 10 minutes.
8. Remove the weight, the glass plate, and the paper toweling.
9. Rewet the blotting paper thoroughly with distilled water and gently lift it off the gel surface.
10. Wash the gel in distilled water for 15 minutes to remove residual fixative and ampholytes.
11. Dry the gel completely in a forced hot air oven (50ºC-55ºC). Drying usually takes less than ½ hour.
12. Stain with Coomassie® or Crowle's Double Stain for 15-30 minutes.
13. Float the gel face down into the stain so that precipitated stain will not settle on the gel surface.
14. Remove the gel, rinse it with distilled water.
15. Place the gel in destain for 3 minutes.
16. Rinse again briefly in distilled water. (This applies for either stain used).
17. Clamp the GelBond® Film, gel side out, onto a glass plate to prevent curling during drying.
18. Dry the gel either in a forced hot air oven (50ºC-55ºC) for approximately 15 minutes or dry at room temperature overnight.
    NOTE: Gels will crack if overdried. The gel can be read, stored or otherwise used without further treatment.

Silver Stain

A modified silver stain procedure has been developed for use with agarose gels cast on GelBond® Film. After electrophoresis the gels are fixed, press blotted and completely dried down *before* staining. Perform all fixing and staining steps in acid-cleaned (50% HNO_3) glassware. All washes are done with constant agitation in a volume of at least 250 ml (gel volume:reagent volume = 1:22). Coomassie® Brilliant Blue stained gels may be silver stained after drying. In this case proceed to the glutaraldehyde pretreatment step (below).

1. Fix the gel for 10 minutes.
2. Place the gel on a paper towel, gel side up.
3. Place a single sheet of Whatman® 3MM Blotting Paper, wetted with distilled water, on the gel surface.
4. Overlay the blotting paper with six layers of absorbent paper toweling and weight it down with a 1 kg-2 kg weight for 30 minutes.
5. Remove the weight, the glass plate, and the paper toweling.

6. Rewet the blotting paper thoroughly with distilled water and gently lift it off the gel surface.
7. Wash the gel in distilled water for 15 minutes to remove residual fixative and ampholytes.
8. Clamp the GelBond® Film, gel side out, onto a glass plate to prevent curling during drying.
9. Dry the gel in a forced hot air oven (50ºC-55ºC) for approximately 15 minutes or until dry.
   NOTE: Gels will crack if overdried.
10. Soak the dried gel in 2% glutaraldehyde for 10 minutes, followed by a 10 minute wash in distilled water using mild agitation.
11. Soak the gel for 10 minutes in 0.01% DTT(dithiothreitol), followed by a 10 minute wash in distilled water using mild agitation.
12. Pour an equal volume of Silver Stain Solution B *into* vigorously stirring Silver Stain Solution A (75 ml B and 75 ml A for each gel to be stained).
13. Transfer the solution to an acid-cleaned glass dish containing one gel.
14. Stain the gel 10 minutes with gentle agitation. (There will be some background).
15. Transfer the gel to a stop bath containing 1% acetic acid and gently agitate for 5 minutes.
16. Rinse the gel in distilled water, wipe any silver deposits from the back of the gel and allow to air dry.

Sample Pretreatment

Successful isoelectricfocusing in part depends upon the condition of the sample. Situations such as high salt content, particularly in cases of high sample loading, or insolubility should be addressed before the sample is loaded onto the gel.

- Dialyze the sample against distilled water, 1% glycine or 0.05 M-0.1 M ammonium bicarbonate solution for samples with high salt content.
- To dissociate protein aggregates, or subunit assemblies and to unfold peptide chains: Add urea to a final concentration of 4 M to 9 M to both the sample and the gel.
- Samples that are hydrophobic or poorly soluble at or near their pI point.
- Add either nonionic or zwitterionic detergents to the sample and the gel at a final concentration of 0.05-1.0%.

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

  Zwitterionic detergents-
  CHAPS®
  (available from Sigma Chemical Co., St. Louis, MO)
  Zwittergent® 3-14
  (available from Calbiochem/Behring, LaJolla, CA)

Press Blot Transfer of Proteins from IsoGel® Agarose

Press blot transfer is a quick method of removing proteins from agarose gels. The procedure involves overlaying the gel with a buffer-soaked nitrocellulose membrane covered by a thick filter pad and several layers of dry paper toweling. The assembly is then covered by a glass plate. After just 1 ½ minutes of press blot approximately 20% of the proteins are transferred from the gel to the membrane. Up to 85% transfer can be achieved with a 35-40 minute blotting time.

Transfer of proteins from the IEF gel to nitrocellulose:

1. Prepare a solution of Tris-saline buffer, pH 7.5:
   50.0 g Tris-HCLl0.94 g Tris-Base58.48 g NaCl
   Fill to 2 liters with distilled water.
2. Cut one piece of nitrocellulose membrane and thick filter paper to the same dimension as the IEF gel. (Wear gloves to prevent contamination by extraneous proteins).
3. Evenly wet the nitrocellulose membrane in the Tris-saline buffer by holding one end of the membrane with smooth tipped forceps and lowering the other end into the buffer container, dropping the membrane flat on the buffer surface. The membrane must be completely saturated with buffer.
4. After isoelectric focusing is complete, remove the IsoGel® Agarose IEF gel from the chamber and place it on a flat surface, gel side up.
5. Place the buffer-soaked nitrocellulose membrane onto the gel surface. Avoid trapping air bubbles between the gel and the membrane.
6. Place one piece of buffer soaked filter pad on top of the nitrocellulose membrane followed by three layers of dry paper toweling.
7. Cover with a glass plate slightly larger than the gel surface. No other weight is used.
8. Press blot for 1 ½ minutes.
9. Remove glass plate and discard the paper toweling and filter paper.
10. Transferred proteins can be detected on the membrane and on the gel by standard methods. Differential detection may be done.

Preparative Isoelectric Focusing

Separation of relatively large amounts of biologically active macromolecules is possible by isoelectric focusing in agarose. Typical high yield recoveries of applied proteins are obtainable with retention of biological activity. This preparative isoelectric focusing procedure is based on the work of Cantarow, et al.

As much as 120.0 mg of protein can be focused in 9.5 ml (0.75 cm x 10 cm x 11.5 cm) of 1% IsoGel® Agarose containing 2.5% ampholytes. Follow the procedure described under IsoGel® Plate Preparation for gel casting adding the sample to the IsoGel® Solution at 60ºC.

After gelation, allow the gel to equilibrate before beginning isofocusing by placing it on the 10ºC cooling platen for 10 minutes.

Run the isoelectric focusing under standard conditions. To recover proteins from the gel use a spatula to cut 5 mm wide slices from the gel, stripping the agarose from the GelBond® Film. Place the agarose strip in a 5-cc plastic syringe fitted with an 18-gauge needle. Macerate the gel slice by expelling it into a clean tube. Add 4.0 ml of phosphate-buffered saline (PBS) to the macerated gel. Cover the tubes and place them on a test tube rocker for 16 hours at 4ºC. Centrifuge the mixture for one minute at 100 x *g* and separate the supernatant from the gel using a serum separator.

Immunofixation/Immunoperoxidase or Autoradiography

Detection of separated species can be accomplished by protein stains or by overlaying gels with specific antibody solutions coupled to enzymes that will eventually produce a visible end product. The antibody-peroxidase conjugate

system or autoradiography with ^{125}I labeled antibody are frequently employed for this purpose.

Immunoperoxidase labeling of focused proteins is done according to Saravis, et al. After focusing, fixing and drying the IEF gel, treat the gel as outlined below:

Immunoperoxidase Staining/Avidin-Biotin Modification:

1. Soak the gel in 3% hydrogen peroxide for 10 minutes.
2. Rinse with distilled water.
3. Soak the gel in 2.28% periodic acid for 5 minutes.
4. Rinse with distilled water.
5. Soak the gel in 0.02% sodium borohydride for 2 minutes.
6. Rinse with distilled water.
7. Place the gel in Tris-saline 0.05 M, ph 7.6 for 10 minutes.
8. Incubate the gel with 1:5 normal serum for 10 minutes (same species as secondary antibody).
9. Incubate the gel for 2-4 hours at room temperature with anti-(primary antibody).
10. Rinse the gel with 0.15 M phosphate-buffered saline (PBS), pH 7.4, for 1 hour at room temperature.
11. Incubate the gel with secondary* antibody (e.g. Goat anti-mouse IgG). If the avidin/biotin modification is used, proceed with steps 12 and 13 using biotinylated reagents (marked with * in steps 11 and 15).
12. Rinse the gel with 0.15 M PBS for 1 hour at room temperature.
13. Incubate the gel with avidin solution (40 mg/gel) in PBS for 1 hour at room temperature (stock solution of avidin is usually 20 mg/ml).
14. Rinse the gel with 0.15 M PBS for 1 hour at room temperature.
15. Incubate the gel with horseradish-peroxidase* (33 mg/gel) for 2 hours at room temperature.
16. Rinse the gel with 0.15 M PBS for 1 hour at room temperature.
17. Incubate the gel with diaminobenzidine (0.15 mg/ml) and hydrogen peroxide (0.03%) in 0.01 M Tris-0.15 M NaCl for 1-17 hours at room temperature.
18. Rinse the gel in PBS and dry.

Direct Tissue Isoelectric Focusing (DTIF)

This method employs the application of tissue or cell pellets directly onto the surface of the IEF gel without concentration of samples, dialysis to remove salts, or the salt extraction of soluble proteins from tissue. Briefly, a tissue slice is draped into the open slot of an application mask on the gel surface, refocused for 10-15 minutes at low voltage to allow sample uptake, and then completely removed. Focusing is continued using standard methods. After focusing is complete, the gel is fixed, stained and dried using standard conditions.

DTIF allows more soluble protein per milligram of tissue to enter the gel than is recoverable by extraction procedures and minimizes denaturation of biologically active proteins that can be damaged by extraction.

Urea in IsoGel® IEF Gels

Urea is a common additive in the focusing of membrane and aggregate proteins. High concentrations of urea inhibit agarose gelation and the gels that are formed are much weaker and more fragile than standard IEF gels. Therefore, it is best not to blot an agarose-urea gel. After gelation, gels containing urea should be refrigerated overnight before use.

Gel Preparation

Agarose-urea gels should be prepared by first dissolving the agarose in a minimum volume of water or buffer, then cooling to slightly below 50ºC before stirring in solid urea or a high concentration solution of urea. Once the urea has dissolved, adjust to the desired total volume with water or buffer. Cast high urea concentration gels (7 M-9 M) and refrigerate overnight. If gels are quick-chilled by placing them in a freezer for 15-20 minutes, urea crystals may be removed by allowing the gel to sit at room temperature for a few minutes.

Gel strength may be enhanced by increasing the IsoGel® Agarose concentration of the gel. If a satisfactory gel cannot be obtained by the above procedure, cast an aqueous IsoGel Agarose gel, and then allow it to equilibrate with a urea/ ampholyte solution.

CAUTION: Avoid exposing urea to high temperatures. Urea solutions decompose to ammonia and carbon dioxide with excessive heat. Cyanate formation and carbamylation of other gel components, such as ampholytes, may also occur.

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