GE Healthcare

Instructions 52-1536-00 AF

Agarose IEF

Introduction

Agarose IEF is a modified agarose suitable for isoelectric focusing.
It is recommended for use with Pharmalyte and Ampholine preblended pH intervals.

Agarose IEF is a highly purified agarose. Any remaining residual quantities of carboxylate and sulphate ester groups are balanced by chemical means. This reduces the electroendosmosis to a minimum with no gradient drift in the neutral pH region.

Casting Agarose IEF onto GelBond film makes the gel easy to handle. It will not float off during the fixing and staining processes. The gel is optically clear for scanning under visible light, and the results can conveniently be stored in an ordinary file.

The experimental conditions for using Agarose IEF described in these instructions have been developed in our laboratories for use with Pharmalyte and Ampholine carrier ampholytes.

Used correctly, Agarose IEF will give excellent results consistently and easily.

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1. Materials

Product	Code No.
Multiphor II electrophoresis unit	18-1018-06
Electrophoresis Power Supply EPS 3500	19-3500-00
MultiTemp III, thermostatic circulator	
thermostatic circulator for 110 V	18-1102-77
thermostatic circulator for 220 V	18-1102-78
Roller	80-1106-79
Agarose IEF, 10 g	17-0468-01
PhastGel Blue R (40 tablets)	17-0518-01
GelBond film, 124 × 258 (50/pkg)	80-1129-32
Sample Application Syringe, 15 ul	80-1106-48
IEF sample applicator pieces. (200/pkg)	80-1129-46
EPH/ IEF sample Application Foil	
24 sample 2–4 ul (5/pkg)	80-1129-47
Filter paper 104 × 253 mm (500/pkg)	80-1129-52
Pharmalyte 3–10 (25 ml)	17-0456-01
Pharmalyte 4–6.5	17-0452-01
Pharmalyte 5–8	17-0453-01
Pharmalyte 8–10.5	17-0455-01
Pharmalyte 2.5–5	17-0451-01
Pharmalyte 4.2–4.9	17-0562-01
Pharmalyte 4.5–5.4	17-0563-01
Pharmalyte 5–6	17-0564-01
Pharmalyte 6.7–7.7	17-0566-01
Ampholine, preblended pH 3.5–9.5	80-1127-15
Ampholine, preblended pH 4.0–6.5	80-1127-17
Ampholine, preblended pH 5.0–8.0	80-1127-19

2. Reagents

Electrode solutions

Table 1. Recommended electrode solutions for Agarose IEF gels.

pH interval	Anode (+) solution	Cathode (-) solution
2.5–5	0.01 M H_2SO_4	1 M NaOH
4–6.5	0.01 M H_2SO_4	1 M NaOH
4.2–4.9	0.04 M (DL) Glutamic acid	1 M NaOH
4.5–5.4	0.04 M (DL) Glutamic acid	1 M NaOH
5–6	0.04 M (DL) Glutamic acid	0.2 M L-Histidine
5–8	0.01 M H_2SO_4	1 M NaOH
6.7–7.7	0.25 M HEPES	0.1 M NaOH
8–10.5	0.20 M L-Histidine	1 M NaOH
3–10	0.01 M H_2SO_4	1 M NaOH
3.5–9.5	0.01 M H_2SO_4	1 M NaOH

Fixing solutions

5% Sulphosalicylic acid

10% Trichloroacetic acid

in distilled water

Destaining solution

35% Ethanol

10% Acetic acid

in distilled water

Staining solution

Dissolve 1 tablet PhastGel BlueR in 200 ml destaining solution by heating to 60 °C. Stir constantly and filter before use.

3. Gel casting

Assemble the mould, follow the gel casting instructions in the Multiphor II Electrophoresis System User Manual (Code No. 18-1103-43).

Prepare the gel solution:

To cast one gel 0.5 × 125 × 250 mm, prepare the following solution:

Note: Ampholine or Pharmalyte is added after dissolving the agarose

0.2 g Agarose IEF

2.4 sorbitol

18.0 ml of distilled water

1.3 ml of Pharmalyte or Ampholine

Mix the agarose and sorbitol in water, in a 50 ml conical flask. Dissolve the agarose by heating the mixture in a boiling water bath, or by using an electrically heated magnetic stirrer. When the agarose is dissolved (after about 10 minutes), decrease the temperature of the water bath to around 75 °C. Add Pharmalyte or Ampholine and mix thoroughly. Use a syringe to fill the mould. Allow the gel to cool down to room temperature. To obtain optimal gel strength store the gel for 1 hour at 4 °C or overnight at room temperature. Store the gel in the mould or in a humidity chamber e.g. a closed plastic box with moist paper towelling.

4. Running conditions

1. Connect the Multiphor II to MultiTemp III. Switch on MultiTemp III 15 minutes before starting the electrophoresis and set the temperature recommended in table 2 (standard running temperature is 10 °C).
2. Pipette 2 ml of distilled water onto the cooling plate. Place the Agarose IEF gel on the cooling plate, making sure no air bubbles are trapped under the gel. Remove excess water with paper towelling.
3. Soak the electrode strips in the appropriate electrode solution (see table 1)

   Blot the strips on filter paper for about one minute to remove excess liquid. Apply the anode and cathode strips on the respective gel side. Any moisture expelled during the run may then be adsorbed by the strips.
4. Apply the sample using IEF sample application pieces. Up to 25 µl can be applied using the IEF sample application pieces. Alternatively, use Sample application strips.
5. Set the running conditions according to table 2.

Table 2. Recommended focusing conditions for using Ampholine and Pharmalyte in Agarose IEF gels. Inter-electrode distance of 100 mm, gel length of around 200 mm and gel thickness of 0.5 mm. For thinner gels reduce the power in proportion to the gel thickness.

pH interval		Voltage (V)	Current (mA)	Power (W)	Time (hours)	Volthours (Vh)	Running temp.(°C)
2.5–5		600	150	3	3	1500–2000	10
4–6.5		1500	150	8	1.5	1500–2000	10
5–8		1500	150	8	1.5	1500–2000	10
8–10.5		600	150	3	3	1500–2000	10
4.2–4.9	c	1500	150	3	0.4	400	20e
	d	1500	150	8	1.5	1600	20e
4.5–5.4	c	1500	150	3	0.4	400	20e
	d	1500	150	8	1.5	1600	20e
5–6	c	2000	150	3	0.4	400	10
	d	2000	150	8	1.4	1600	10
6.7–7.7	c	1500	150	3	0.4	400	20e
	d	1500	150	8	1.4	1600	20e
3–10		1500	150	8	1.5	1500–2000	10
3.5–9.5		1500	150	8	1.5	1500–2000	10

c: Prefocusing conditions. To obtain optimal results, gels must be prefocused. After prefocusing, samples are applied 1.5–2 cm from the cathode.
d: Focusing conditions.
e: Optimal temperature for specific applications (4.2–4.9: α1-antitrypsin, 4.5–5.4: Gc-globulin, 6.7–7.7: haemoglobin). For other samples, use 10 °C.

6. Remove the sample applicators after 45 minutes.

5. PhastGel Blue staining

1. Immediately after the run is completed, put the gel in fixing solution for 30 minutes.
2. Wash the gel in two lots of destaining solution, each for 15 minutes.
3. Place three layers of filter paper carefully on top of the gel, followed by a glass plate and a weight of about 1 kg. Remove everything after about 15 minutes and dry the gel with a hair dryer.
4. Place the dried gel in staining solution for 5 to 10 minutes.
5. Destain the gel in destaining solution until the background is clear (15 to 30 minutes).
6. Finally, dry the gel again with a hair dryer.

6. Silver staining

Low concentrations of proteins are detected by silver staining. Silver staining can be applied after staining with PhastGel Blue.

See reference, Anal Biochem. 130 (1983) 353–358. Willoughby E.W. and Lambert A.

7. Zymogram techniques

Enzyme activity in the focused gel is detected by zymogram techniques. Once the pattern has developed, it is often possible to preserve the results, especially if the visible product is insoluble e.g. (MTT) Formazan. The gel is pressed under filter paper as previously described and then washed in distilled water and dried again. See reference. Anal. Biochem 203, (1992)1–21. Gabriel O. and Gersten D.M.

8. Immunotechniques

Following IEF, proteins may be identified by immunofixation techniques. For example, most serum proteins can be identified by using cellulose acetate strips soaked in the specific antiserum. See reference Clin. Chem. 22/4 (1976) 497–499. Ritchie R.F. and Smith R. Agarose IEF is also a suitable medium for IEF followed by crossed immunoelectrophoresis.

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