INSTRUCTION MANUAL

FocusGel 6-11

Precast Horizontal Gels for Isoelectric Focusing (Flatbed IEF)

(Cat. No. 43329, 43333)

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1. FocusGel 6-11

1.1. General information

FocusGels are pre-polymerized under optimized conditions to produce a matrix optimal for isoelectric focusing. FocusGels are thin gels (0.65 mm) with a gel concentration (T) of 5 % and cross-linking (C) of 3 %.
Catalysts as well as other toxic and non-polymerized compounds are washed from the matrix resulting in gels that are non-toxic. The gel contains a carrier ampholyte cocktail designed to achieve an optimal pH gradient.
No electrode solutions and electrode strips are required and the electrodes are placed directly on the gel surface.

1.2. Storage conditions

Recommended storage temperature is 2 °C to 8 °C (35 °F – 46 °F).
Do **not** freeze the gels or leave them at room temperature for longer periods as this may impair their separation properties.

If stored at the recommended temperature at least useable until: see expiry date on package.

2. Performing isoelectric focusing

2.1. Sample preparation

Using subsequent **Coomassie Blue staining**; add **1 ml** distilled water to approximately **1 µg** of protein.

Using subsequent **silver staining**; add **5 ml** of distilled water to approximately **0.1 µg** of protein.

Serum and CSF samples: Sample preparation and dilution is more critical than usual; see special instructions in section 2.3.

2.2. Handling of the gel

Open the bag with scissors and carefully remove the gels. Then remove the protective cover-film from the gel surface. Keep the cover film as it will serve as a protective sheet later. The gel is ready for use.

Spread 2 ml Cooling fluid onto the cooling plate of the focusing chamber to ensure good cooling contact (Fig. 1).

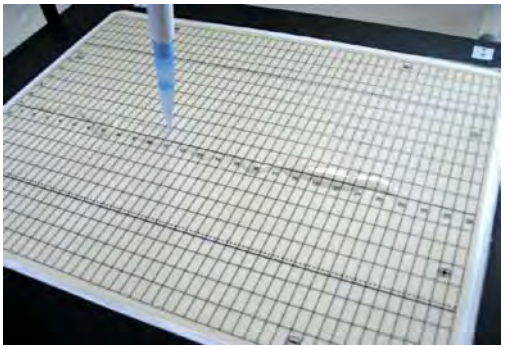

Fig. 1

Important: To avoid water condensation on the gel surface at this time, do not yet switch on chiller or set cooling tubing to “bypass”.
Place the gel (gel side-up) on the center of the cooling plate (Fig. 2), avoid trapping any air bubbles. For the FlatTop Large match the edges of the backing with the lines 4 and 16. For the GE Healthcare Multiphor II the gel edges should match with the lines 3 and 15.

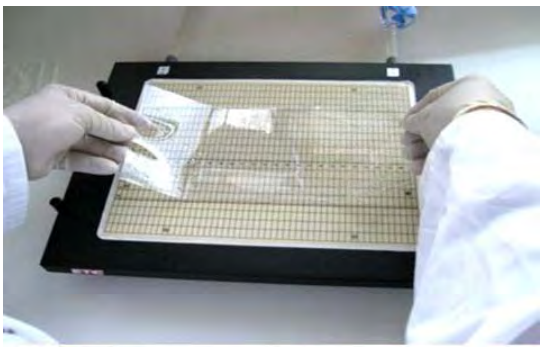

Fig. 2

2.3. Isoelectric focusing of liquor and serum

2.3.1. Sample Dilution

All sera should be diluted first: 5 µl serum + 495 µl Sample Diluter.

IMPORTANT:
All dilutions (incl.the 1:100 dilution-step of the sera) must be performed with the Sample Diluter. **Do not use PBS (Phosphate-Buffered Saline) or 0.9 % NaCl!**

The IgG-concentrations are determined with a nephelometer! All cerebrospinal fluids are directly adjusted to the same protein concentration like the sera.

Sample dilutions for different visualization procedures of oligoclonal bands

General silver staining
This method should be taken as routine staining. It is not IgG sensitive, all sample proteins are detected.

- Best resolution and sensitivity
- Adjust sera and CSF to 20 mg/l IgG

Immunofixation with fluorescence imaging

- IgG selective staining, very sensitive
- Needs a Fluorescence Imager
- Adjust sera and CSF to 15 mg/l IgG

Immunofixation followed by silver staining

- IgG selective staining, very sensitive
- Needs an overnight step (washing out of the excess antibodies)
- Adjust sera and CSF to 5 mg/l IgG

Blotting with immunostain

- IgG selective staining
- Needs an aditional blotting step
- Adjust sera and CSF to 20 mg/l IgG

2.3.2. Sample application

FocusGel 24S und 40S:
For serum and CSF the position of the pre-formed wells is optimized for anodal application in a pH gradient 6-11 (Fig. 3). This well position might also be suitable for other sample types. The gels can be turned around for cathodal application.

Fig. 3

Apply 25 µl (24S) or 12 µl (40S) of serum and cerebrospinal fluid alternating.

Note: All wells must be filled with liquid; i.e. with sample or IgG sample diluter.
Apply 25 µl (24S) or 12 µl (40S) of the Sample Diluter to non-used slots.

2.4. Isoelectric Focusing

- **Electrode positioning**
  Clean the platinum wires with moist tissue paper before (and after) IEF runs.
  Move the platinum electrodes to the correct positions over the edges of the gel.
  Lower the electrode holder onto the gel surface.
  IMPORTANT: The platinum wires should rest directly on the gel edges and not on the support film.
  Apply the samples, close the safety lid, and start focusing

  Note: There is no requirement to use electrode strips or buffers. Those must not be used.

- **Temperature**
  Isoelectric focusing has to be performed at a defined constant temperature because the pH gradient and the isoelectric points depend on the temperature. Native IEF is usually done at 10 °C. Than the pH gradient matches with most of the commercially available pI standards.

- **Power supply settings**
  During isoelectric focusing the electric resistance of the gel is changing considerably. For some samples it is beneficial to start with prefocusing of the pH gradient before sample application (**not recommended for CSF analysis of serum and liquor**). In this case add a Step 0.1 with the following settings:

STEP	SET	SET	SET	Time	Process
0.1	1000 V	50 mA	10 W	20 min	Prefocusing without sample

It is recommended to use a programmable power supply, e. g. SERVA BluePower 3000x4 Power Supply (BP-3000x4).

STEP	SET	SET	SET	Time	Process
1	500 V	30 mA	10 W	30 min	Sample entrance
2	1500 V	18 mA	20 W	1 h 30 min	Focusing
3	2000 V	15 mA	25 W	30 min	Band sharpening

For a half gel apply the same voltage (V), half of the current (mA) and power (W).

IMPORTANT: These running conditions are only valid for aluminum oxid ceramics cooling plates! If the cooling plates are made from metal and/or glass:
Do not apply more than 10 W!

3. Staining

3.1. Hot Coomassie Blue G 250

3.1.1. Stock solutions

- 20 % Trichloroacetic acid: Dilute 52 ml of 77 % (w/v) TCA to 200 ml
  To prepare a 77 % (w/v) solution of TCA we recommend adding 300 ml of water to a 1 kg bottle of TCA and dissolve completely.

- Solution A: 0.2 % (w/v) $CuSO_4$ / 20 % (v/v) acetic acid
  (2 g of $CuSO_4$ in 1L of 20 % (v/v) acetic acid)

- Solution B: 0.04 % (w/v) Coomassie Blue G 250 in 60 % (v/v) methanol
  (0.4 g Coomassie G 250 in 1L, 60 % (v/v) methanol)

- Solution C: 50% (v/v) Methanol

3.1.2. Staining protocol

- ***Fix:*** 15 min in 200 ml 20 % TCA (at room temperature)

- ***Wash****:* 2 × 1 min in 200 ml wash solution (*mix equal amounts of A and C*)

- ***Stain:*** 45 min in 200 ml of staining solution (*mix equal amounts of A and B*). Heat (50°C) solution while stirring (Fig. 4). *Suitable steel trays with lids and grids are listed in section 5.*

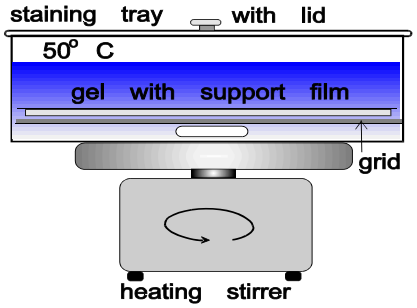

Fig. 4

- ***Wash:*** 2 × 5 min in 200 ml wash solution (*mix equal amounts of A and C*)
- ***Destain:*** 2-3 × 15 min in wash solution (in a tray), A and C
- ***Impregnate:*** 5 min in 200 ml 5 % (w/v) glycerol
- ***Dry:*** air-dry (leave at room temperature)

3.2. Silver staining

There are two concepts for sensitive silver staining:

- Silver nitrate protocols
- Ammoniacal protocols with silver diamine

SERVA offers a special silver staining kit , developed and optimized for detection of oligoclonal IgGs in cerebrospinal fluid (CSF): **SERVA CSF Silver Staining Kit (Cat. No. 43398)**. This staining kit allows protein detection on polyacrylamide gels with high sensitivity.

Because there are many FocusGels users, who do not want to change their standard procedure, this instruction contains also the protocol for an alternative ammoniacal staining.

3.2.1. Protocol for ammoniacal silver staining

Step	Solution	Volume	Time
1. Fixing	20 % (w/v) trichloroacetic acid see above (CBB staining)	200 ml	45 min
2. Washing	$H_2O_{dist.}$	200 ml	5 min
3. Rinsing I	50 % (v/v) methanol / 10% (v/v) acetic acid	200 ml	40 min
4. Rinsing II	5 % (v/v) methanol / 7% (v/v) acetic acid	200 ml	20 min
5. Incubation	2.5 % glutaraldehyde (gels may be kept overnight at this stage)	200 ml	15 min
6. - 9. Washing	$H_2O_{dist.}$	4 × 200 ml	20 min 15 min 10 min 10 min
10. Silvering *(Freshly prepared solutions)*	Solution 1: Dissolve 250 mg $AgNO_3$ in 1 ml $H_2O_{dist.}$ Solution 2: 40 ml H_20 dist + 4 ml NaOH (1 M) + 1.5 ml NH_3 (25 %) Drop Solution 1 into 2 while stirring, fill up to 200 ml with $H_2O_{dist.}$	200 ml	40 min
11.-12. Washing	$H_2O_{dist.}$	2 × 200ml	1 min 5 min
13. Developing *(Freshly prepared)*	0.0025% (w/v) citric acid + 100 µl formaldehyde in 200 mL with H_2O_{dist}	200 ml	2-5 min **Visual control!** **Set Beep in Step 12: Stop when background turns yellow.**
14.-16. Stopping & preserving	10 % (v/v) ethanol, 1 % (w/v) glycine, 5 (w/v) % glycerol	3 × 200 ml	3 x 10 min

Drying: Air-dry the gel down on the film support, then roll the polyester cover film (supplied with the gel) onto the surface.

To get optimal staining results, use only reagens in p.a. quality and high quality dist. water.

3.2.2. Staining with the SERVA CSF Silver Staining Kit (Cat. No. 43398)

Kit components:

Component	Amount
Fixing Solution I	1 L
Ethanol denatured for Fixing Solution II and Wash Solution	1 L
Fixing Solution III	500 ml
Solution A	2x 55 ml
Solution B	2x 55 ml
Solution C	2x 1 ml
Solution D	2x 1,5 ml
Solution E	5x 2 g

Required solutions:
One gel (12 cm x 25 cm) can be stained using 200 ml of each solution in the various steps.

- Fixing Solution for Fixing step II:
  (30 % (v/v) Ethanol)
  60 ml Ethanol, denatured
  ad 200 ml dH_2O
- Fixing Solution for Fixing step III:
  80 ml Fixing Solution III
  ad 200 ml dH_2O
- Wash Solution:
  (5 % (v/v) Ethanol)
  10 ml Ethanol, denatured
  ad 200 ml dH_2O
- Staining Solution:
  20 ml Solution A
  20 ml Solution B
  ad 200 ml dH_2O
- Developer:
  50 µl Solution C
  200 µl Solution D
  ad 200 ml dH_2O
- Stop Solution:
  (1 % (w/v) Glycine)
  2 g Solution E
  ad 200 ml dH_2O
- Conservation:
  12 ml Glycerol (85 %)
  ad 200 ml dH_2O

Please note:

When using an automated gel stainer, air-bubbles below the gel should be avoided. The gel could be positioned and fixed with the help of magnetic bars.

Step	Solution	Time
1. Fixing I	Fixing Solution I	20 min
2. Fixing II (1)	Fixing Solution for Fixingstep II: 30 % (v/v) Ethanol	10 min
3. Fixing II (2)	Fixing Solution for Fixingstep II: 30 % (v/v) Ethanol	10 min
4. Washing (1)	Wash Solution: 5 % (v/v) Ethanol	10 min
5. Washing (2)		10 min
6. Fixing III	Fixing Solution for Fixingstep III	20 min
7. Washing (1)	Wash Solution: 5 % (v/v) Ethanol	10 min
8. Washing (2)		10 min
9. Rinsing (1)	200 ml dH_2O	10 min
10. Rinsing (2)		10 min
11. Rinsing (3)		10 min
12. Staining	Staining Solution: 20 ml Solution A + 20 ml Solution B ad 200 ml dH_2O	30 min
13. Rinsing (1)	200 ml dH_2O each	2 min
14. Rinsing (2)		5 min
15. Developing	Developer: 50 µl Solution C+ 200 µl Solution D ad 200 ml dH_2O	2 to 3 min (by sight)
16. Stopping	Stop Solution: 1 % (w/v) Glycine	5 min
17. Rinsing (1)	200 ml dH_2O each	5 min
18. Rinsing (2)		5 min
19. Preservation	Preservation solution	5 min
20. Drying	Air-dry the gel, then roll the gel cover sheet supplied with the gel onto the gel surface	Several hours

To get optimal staining results, use only reagens in p.a. quality and high quality dist. water.

4. Trouble shooting for IEF of oligoclonal IgGs

Symptom	Cause	Remedy
Voltage applied, but no current. Samples and their colour remain in the slots.	No internal connection in the chamber. Electrodes have no contact with gel surface.	Check internal cables in the chamber. Lower the electrodes onto the gel surface. Follow the manual carefully.
Lanes have different widths. Sera and CSF stain differently.	Sera and CSF have been diluted wrongly.	Use the Sample Diluter for all sample dilutions. Do not forget the pre-dilution of the sera.
Lanes are not running straight	Not all sample slots filled	Pipet at least 25 µl Sample-Diluter. Do not let any slot empty.
At the cathode the gel is burning.	Salt load for the gel is too high. Electrode solutions and strips were used.	Do not apply solely CSF-samples without sera in between. **Do not use PBS or 0.9 % NaCl for dilution!** **Do not apply electrode strips.**
Silver staining does not function at all	Inadequate reagent quality	Use the recommended quality.
Silver staining has (nearly) no contrast.	Formaldehyde-containing liquids or the citric acid solution older than 1 day.	Check reagents and water quality. Clean the tubings of the Autostainer!

5. Ordering Information

Product	Size	Cat. No.
Reagents		
IgG Sample diluter IEF	100 ml	43336.01
Cooling Contact Fluid	50 ml	43371.01
	3x 50 ml	43371.02
Glycerol from plant	1 L	23176.01
Staining kits and -reagents		
SERVA CSF Silver Staining Kit	1 kit	43398.01
Trichloroacetic acid, 20 % solution	500 ml	36913.01
	1 L	36913.02
Silver nitrate	25 g	35110.01
	100 g	35110.02
Citric acid	500 g	38640.01
	1 kg	38640.02
	5 kg	38640.03
Glycine	500 g	23390.02
	1 kg	23390.04
	5 kg	23390.03
Coomassie® Brilliant Blue G 250	25 g	17524.01
	100 g	17524.02
SERVA Blue G	5 g	35050.01
	25 g	35050.02
	100 g	35050.03
Equipment		
HPE™ Tower System		HPE-TS1
HPE™ FlatTop Tower		HPE-T01
HPE™ Cooling Unit		HPE-CU1
SERVA BluePower™ 3000x4 Power Supply		BP-3000x4
Steel Tray Large + Grid + Lid		HPE-A20
Steel Tray Multi 6		HPE-A21