Isoelectric Focusing with CleanGel IEF for PhastSystem®

CleanGel IEF for PhastSystem (cat. no. 43350) are 0.44 mm thin gels with a gel concentration (T) of 5% and cross-linking (C) of 3%. They are pre-polymerized under optimized conditions to produce a matrix optimal for isoelectric focusing. Catalysts as well as other toxic and non-polymerized compounds are washed from the matrix resulting in gels that are non-toxic. The gels have to be rehydrated with a carrier ampholyte cocktail before use. Like with ready PhastGels no electrode solutions and electrode strips are required and the elec- trodes are placed directly on the gel surface.

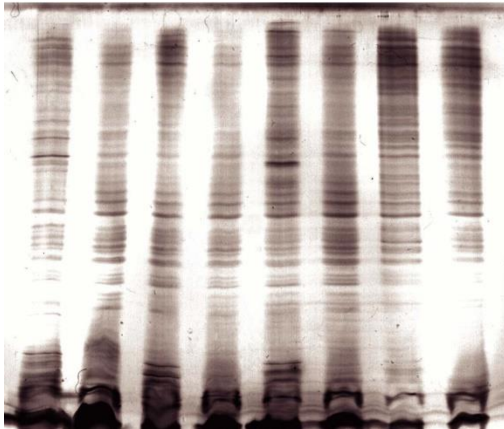


Fig.1: *Isoelectric focusing of serum and cerebrospinal fluid of 4 patients on PhastSystem® with* CleanGel IEF for PhastSystem*. Detection of proteins with silver staining. S=serum, C=cerebrospinal fluid (CSF)*

Always wear powder free disposable gloves.

Rehydration of the gel

1. Preparation of the rehydration solution containing Servalyte mixture + distilled water.
2. Pipet 800 µL of this solution into the GelPool (trays for PhastGels).
3. Lay the dry gel onto the surface of the solution without catching air bubbles

Important !
Remove the cover film from the dry gel.
Place the gel layer side onto the liquid.
Cover the GelPool with a glass plate (*in order to avoid loss of liquid, particularly in dry and warm rooms*).

4. After 5 minutes lift the gel and lay it down again without catching air bubbles
5. Repeat this every 20 minutes, after 2 hours the gel can be used
6. Take the gel out of the GelPool (there should remain less than a rest of 50 µL in the GelPool). Dry the gel surface with the edge of a very clean filter paper.

Important! *The gel surface has to be completely dry !!*

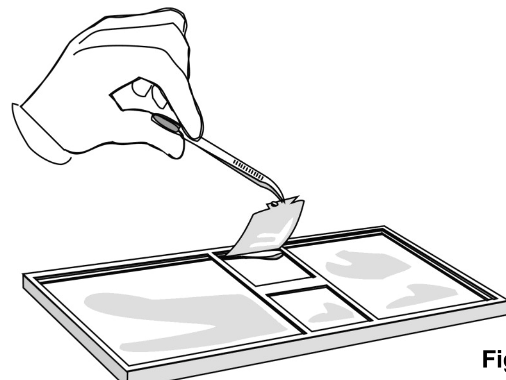


Fig. 2

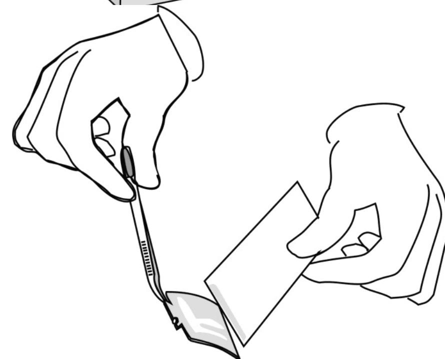


Fig. 3

Isoelectric Focusing

The electrodes of the PhastSystem must be carefully cleaned after (using a toothbrush and H_2O_{dist}) and carefully dried before each run: This is optimally performed by laying them on a tissue paper for several minutes.

Place the gel with 50 µL Cooling Contact Fluid (or kerosene) onto the gel bed of the PhastSystem without air bubbles (the amount of fluid should be as low as possible).

Sample Application

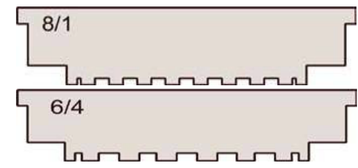


Fig. 4

When sample COMBS are employed (fig. 4)

1. Place the PhastGel IEF Gel Cover into the chamber
2. Lower the electrodes after the gels have been placed onto the gel bed, and start the programme
3. Fill the sample comb 6/4 with 4 µL of each sample (pipette directly into the slots)
4. After the alarm interrupt the programme with PAUSE and insert the sample comb
5. Press CONTINUE for the rest of the programme.

When sample applicator STRIPS are used (fig. 5):

1.Lower the electrodes after the gels have been placed onto the gel bed, the strips - the grips orientated to the gel surface and to the anode - are applied directly at the anodes (max 2 mm distance), they are slightly pressed down with the forceps. The strips are not leaking when a small amount of silicone is applied to the contacting side.

Important! *The gel surface must be dry, the applicator strips must be dry and free of dust. The applicator strips are applied **before** pre-focusing, because during focusing a groove structure develops on the surface which can cause the samples to flow together.*

2. Start pre-focusing

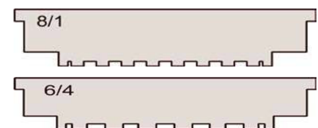
3. After the second step has been reached, interrupt the programme with PAUSE and pipette the samples(**10 µL**, do not touch the applicator strip!).

4. Press CONTINUE for the rest of the programme

Separation programmes

Step	V	mA	W	°C	Vh
.1	1000	3.5	3.0	10	75
.2	200	3.5	3.0	10	25
.3	1000	3.5	3.0	10	100
.4	1500	3.5	3.0	10	350
.5	700	3.5	3.0	10	0

Tab. 3: Programme for Sample applicator **STRIP** (set extra alarm step .1, 73 Vh)



Tab 4: Sample applicator **COMBS**

“sample applicator down at” .2 0 Vh
“sample applicator up at” .3 0 Vh

Step	V	mA	W	°C	Vh
.1	500	2.5	3.0	10	15
.2	200	2.5	3.0	20	25
.3	500	3.5	3.0	10	20
.4	1000	3.5	3.0	10	80
.5	1500	3.5	3.0	10	350
.6	700	3.5	3.0	10	0

Silver Staining

Either use the PlusOne Silver Staining Kit (Protein) from GE Healthcare and the original IEF staining programme, or the SERVA CSF Silver Staining Kit (SERVA 43394.01) with a modified programme.

Important! *Optimize the development time of Step 13: It should be between 2 and 4 min: Control this during the first development. Recommended amount of Formaldehyde in the developer: 50 µL.*
After the last Step (14) treat the gel 10 min with 2% glycerol.

Tab. 6: Tab. 6: Silver staining programme for the GE PlusOne Kit

Step	Solution (min)	Recipe	In	Out	Temp °C	Time
.1	fixing	20 % (w/v) TCA	1	9[1]	50	6
.2	rinsing	10% Ethanol, 5% Essigsäure	2	0	50	3
.3	rinsing	10% Ethanol, 5% Essigsäure	2	0	50	5
.4		75 mL ethanol, 1.25 mL glutardialdehyde, 10 mL sodium thiosulphate, fill up to 250 mL with distilled water, add 1 sachet sodium acetate, use magnetic stirrer	3	9[1]	50	6
.5	rinsing	10% Ethanol, 5% acetic acid	2	0	50	3
.6	rinsing	10% Ethanol, 5% acetic acid	2	0	50	5
.7	washing	Distilled water	4	0	50	2
.8	washing	Distilled water	4	0	50	2
.9	silver	25 mL silver nitrate (2.5%), fill up to 250 mL with distilled water, use magnetic stirrer				
.10	washing	Distilled water	4	0	30	0.5
.11	washing	Distilled water	4	0	30	0.5
.12	washing	Distilled water	4	0	30	4*)
.13	develop	add slowly the content of 1 sachet sodium carbonate to 250 mL distilled water, use magnetic stirrer. Add 75 µL formaldehyde (37%) to 150 mL developer.				
.14	stop	add slowly content of 1 sachet EDTA-Na_2 to 250 mL distilled water, use magnetic stirrer	8	0	30	5

Preserving: 10 min in 2% glycerol[2]

[1]) Please consult local regulations for information on proper disposal

[2] Is not really necessary; only for drying the gel: this keeps the gel flexible and crystall-free. Should be available in the laboratory

*) Different to the PlusOne Silver-Kit manual, average value depents on the water quality.

Tab. 7: Silver staining programme for the SERVA CSF Silver Staining Kit :

Step	Lösung (min)	Rezeptur	In	Out	Temp °C	Time
.1	fixing	20 % (w/v) TCA	1	9[1]	50	6
.2	rinsing	10% Ethanol, 5% acetic acid	2	0	50	3
.3	rinsing	10% Ethanol, 5% Eacetic acid	2	0	50	5
.4	washing	Distilled water	4	0	50	3
.5		100 mL distilled water, add 25 mL Solution A 25 mL Solution B, and 25 mL Glutardialdehyde (25%), fill up to 250 mL with distilled water, mix with magnetic stirrer.	3	9[1]	50	6
.6	washing	Distilled water	4	0	50	5
.7	washing	Distilled water	4	0	50	5
.8	washing	Distilled water	4	0	50	5
.9	silver	100 mL distilled water, add 5 mL Solution C and 25 mL Solution B, fill up to 250 mL with distilled water, add 260 µL Formaldehyde (37%), mix with magnetic stirrer,				
.10	washing	Distilled water	4	0	30	
.11			0.5			
.12	washing	Distilled water	4	0	30	4
.13	washing	Distilled water	4	0	30	4
.14	develop	100 mL distilled water, add 25 mL Solution D, fill up to 250 mL with distilled water, add 260 µL Formaldehyde (37%), mix with magnetic stirrer,				
	Stop	100 mL distilled water, add 25 mL glacial acetic acid, fill up to 250 mL with distilled water, mix with magnetic stirrer,	8	0	30	5

Preserving: 10 min in 2% glycerol[2]

[1]) Please consult local regulations for information on proper disposal

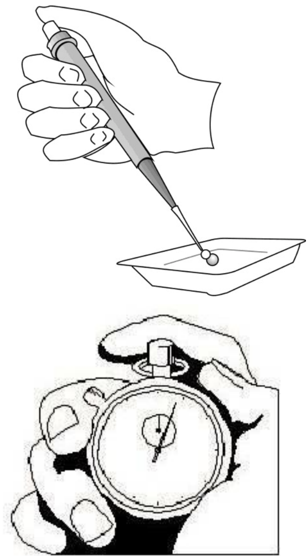
[2] Is not really necessary; only for drying the gel: this keeps the gel flexible and crystall-free. Should be available in the laboratory

*) Depends on the water quality.

Trouble Shooting

Symptom	Cause	Remedy
After silver staining : No bands or only albumin is visible	Reagents from different sources react differently. Also the water quality can differ.	

Silver staining has to be optimized regarding water and chemicals quality in most cases.



Pretesting:

Mix 20 µL of the silver solution ($AgNO_3$ + formaldehyde) and of the developer (+ formaldehyde) in a small dish. The drop has to turn brown immediately, after 10 seconds it has to become black.

a) If this does not happen, order new chemicals ($AgNO_3$, Na_2CO_3, formaldehyde) and/ or check the conductivity of the distilled water.

b) If the two solutions react with each other, then carry out the silver staining.

Optimization of Silver Staining in the Gel:

- Set "Extra Alarm" of the PhastSystem at the end of the step $AgNO_3$ „Silvering". When the automatic silver staining comes to the end of the step "Silvering", staining will stop after the alarm sound (DEV "STOP"). After the chamber is completely emptied of the $AgNO_3$-solution, open the development chamber and take the gel out.
- All further steps are performed in a plastic Petridish under the control of a stop watch:
  Perform the 1st developing step for 0.1 min. Do not forget to shake! Discard the developer , perform the 2nd developing step. Watch the gel, and read from the stop watch the development time for optimal staining of the IgG bands.
- Stop development and impregnate the gel either manually or inside the PhastSystem.
- Subtract 1 minute from the development time measure above (pumping time of

Symptom	Cause	Remedy
Strong, wavy front at the cathode, blurred bands		
Most proteins run at the edge of their lanes.	Application locus is identical to the pI of albumin (albumin blocks the sample entrance).	Place the Sample application strip more to the middle of the gel (1-3 mm). Albumin should leave the application point in direction anode, the Immunoglobulins in direction cathode.
Samples have remained at the position of application, they did not migrate	Uneven or missing electric field, because electrode holders are partly bent	Check regularly, whether electrode holders are straight. If necessary, bend them to their correct place.
Lanes run into each other	Liquid is on the gel surface because of incomplete rehydration	Rehydrate for at least 90 min. If more than 20 µL liquid is left in the GelPool, continue with rehydration for another 15 min.
	Applicator strip does not lay evenly on the gel surface, or on the wrong side.	Press applicator strip along its whole length; Note: The grips must be orientated towards the gel surface. If this does not help: Coat the strip with a very thin layer of Silicone grease
	Too much cooling fluid (or kerosene) is on the cooling plate; cooling fluid (or kerosene) flows onto the gel, this causes the rubber strip to swell.	Apply not more than 90 µL. Soak excess cooling fluid (or kerosene) off with a tissue paper. Always dry the cooling plate carefully after use.
	The band has been displaced during pipetting.	Do not touch the strip with the pipette tip. Rest one hand on the apparatus.

Material Required

CleanGel IEF for PhastSystem	43350.01
Cooling fluid 50 mL	43371.01

1 % Bromophenol blue solution, Bayer Silicone, medium viscous

For Staining: Trichloroacetic acid, ethanol, acetic acid, glycerol,

SERVA CSF Silver Staining Kit SERVA 43394.01

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