INSTRUCTION MANUAL

CSF Analysis Kit for PhastSystem™

Cat. No. 43393

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Studying Oligoclonal IgGs with Isoelectric Focusing using SERVA CSF Analysis Kit on PhastSystem®

This instruction for SERVA CSF Analysis Kit for the PhastSystem (SERVA 43393.01) contains the description for using sample combs as well as sample applicators. Silver staining of all proteins is described as well as immunofixation. It can be stated as a thumb rule: With silver staining of all proteins a minimum of three additional oligoclonal IgG bands in CSF is required for a positive result; with immunofixation one additional oligoclonal IgG band in CSF is required.

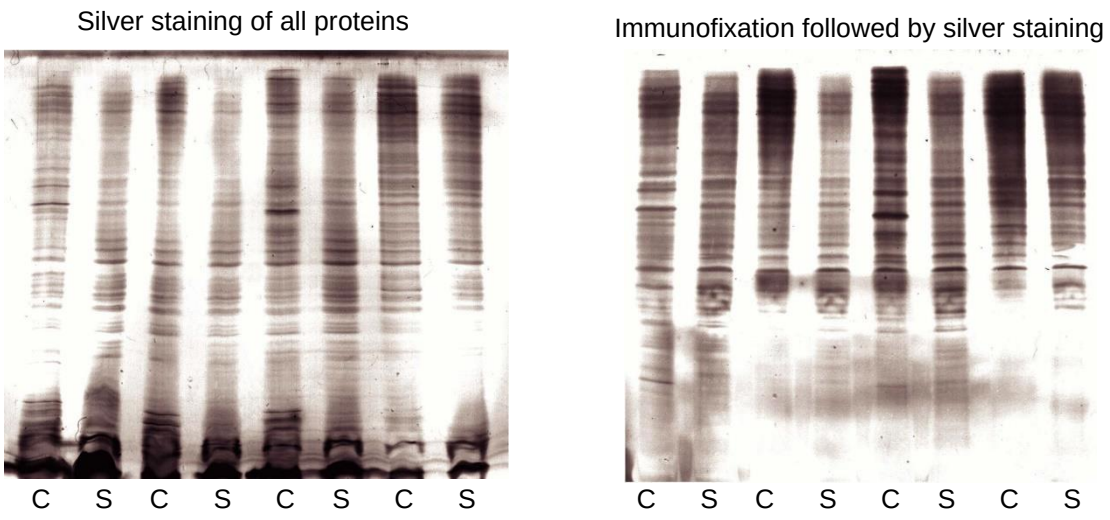


Fig.1: *Isoelectric focusing of serum and cerebrospinal fluid of 4 patients on the PhastSystem® with SERVA CSF Analysis Kit for PhastSystem. Left: Typical result after direct silver staining of all proteins. Right: Typical result after immuno fixation followed by silver staining. S=serum, C=cerebrospinal fluid (CSF)*

Always wear powder free disposable gloves.

Rehydration of the gel

1. Mix 135 µl SERVALYT mixture + 765 µl distilled/ demineralized water
2. Pipet 800 µl of this solution into the rehydration pool (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 rehydration pool *with a glass plate* (*to avoid loss of liquid, particularly in dry and warm rooms*).

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

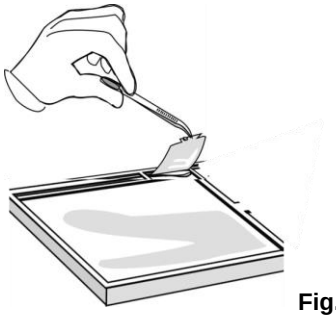


Fig. 2

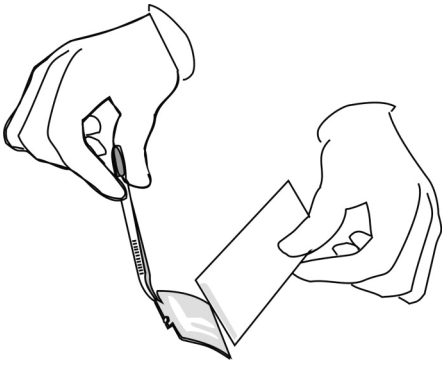


Fig. 3

Dilution of the Serum and CSF Samples:

Solution for Sample dilution:

Mix 0.3 % (w/v) NaCl-solution + 0.003 % Bromophenol blue Na-salt (0.3 g NaCl plus distilled water to 100 ml, add 300 µl of a 1 % Bromophenol blue Na-salt solution). Bromophenol blue in the solution facilitates pipetting.

- The dilutions should be prepared during the rehydration of the gels
- The IgG concentrations are needed in mg/L (determined with a nephelometer)
- For Immuno-fixation use the application with combs. 1µl sample volume (see calculation examples below).
- All samples are adjusted to the IgG target-concentration with 0.3 % NaCl solution (see examples below).

Calculation examples:

Tab. 1: Sample application with combs, IgG target concentration: 20 mg/L, 4 µl respectively 1 µl sample volume

Sample	IgG conc (mg/l)	Sample vol (µl	0.3% NaCl vol (µl)
CSF	40	20	20
Serum (1/100)	80	20	60
CSF	70	20	50
Serum (1/100)	90	20	70
CSF	16	non-diluted	-
Serum (1/100)	103	16	87

Tab. 2: Sample application with strips, IgG target concentration 8 mg/L, 10 µl sample volume

Sample	IgG conc (mg/L)	Sample vol (µl)	0.3% NaCl vol (µl)
CSF	40	8	32
Serum (1/100)	80	8	72
CSF	70	8	62
Serum (1/100)	90	8	82
CSF	16	8	8
Serum (1/100)	103	8	95

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

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 program
3. Fill the sample comb 6/4 with 4 µl of each sample (pipette directly into the slots)
   Use 8/1 comb and pipette 1 µl for immuno-fixation
4. After the alarm interrupt the program with PAUSE and insert the sample comb
5. Press CONTINUE for the rest of the program.

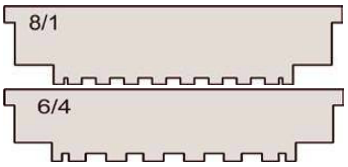


Fig. 4

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

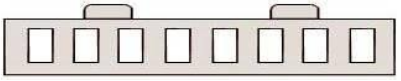


Fig. 5

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 program with PAUSE and pipette the samples (**10 µl**, do not touch the applicator strip!).

4. Press CONTINUE for the rest of the program

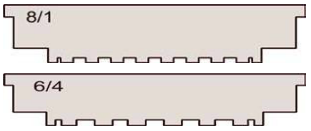
Separation programs

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

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 4: Sample applicator **COMBS**

"sample applicator down at"	.2	0 Vh
"sample applicator up at"	.3	0 Vh
"extra alarm"	.1	13 V



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 program, or the SERVA CSF Silver Staining Kit (SERVA 43394.01) with a modified program.

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.

Immunofixation

(Do not forget to apply only 1 µl sample!)

Additionally required: Polyethylenglycol 6000 (SERVA 39778), PBS (Phosphate Buffered Saline, SERVA 47302), Antibody solution (DakoA424)

Washing solution: Dissolve 30 g PEG 6000 in 1L PBS
Incubation solution: Dilute 15 µl antibody solution with 585 µl washing solution.
Incubation: Pipette 600 µl of the incubation solution into the rehydration pool or a petri-dish and place the gel with its surface onto the liquid (without air bubbles).
Cover it with a glass plate

Incubate for 1 hour at room temperature.

Washing: After the incubation, the non-precipitated proteins must be washed out. This can be performed either in a beaker with washing solution under light stirring overnight, or in the PhastSystem.

Tab. 5: Washing programme for the PhastSystem:

Step	In	Out	Temp °C	Time (min)
.1	4	0	37	90
.2	4	0	37	90
.3	4	0	37	90
.4	4	0	37	90
.5	4	0	37	90

The washing process is improved and slightly accelerated by adding 10 % ethanol.
Directly follow the Silver Staining program in step .4 with glutardialdehyde.

All information concerning rehydration, separation, immunofixation, and staining is valid for the gel and solutions for SERVA CSF Analysis Kit for the PhastSystem and for the PlusOne Silver Staining Kit (Protein).

Silver staining in PhastDeveloper

Tab. 6: Silver staining program, the first three steps are identical for both Silver Staining kits (GE and SERVA):

Step	Solution	Recipe	In	Out	Temp °C	Time (min)
.1	fixing	20 % (w/v) TCA	1	9[1]	50	6
.2	rinse	10% ethanol, 5% acetic acid	2	0	50	3
.3	rinse	10% ethanol, 5% acetic acid	2	0	50	5
↓		Step 4 in table 7 or 8		↓		

Silver Staining after Immunofixation

Apply the silver staining program as usual; after immunofixation start with step 4 as indicated in the table 7 and 8.
All bottles should be cleaned after use in the dishwasher.

Tab. 7: Silver staining program with GE PlusOne Kit :

Step	Solution	Recipe	In	Out	Temp °C	Time (min)
.4	sensitizer	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	rinse	10% ethanol, 5% acetic acid	2	0	50	3
.6	rinse	10% ethanol, 5% acetic acid	2	0	50	5
.7	wash	distilled water	4	0	50	2
.8	wash	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	5	9[1]	40	10
.10	wash	distilled water	4	0	30	0.5
.11	wash	distilled water	4	0	30	0.5
.12	wash	distilled water	4	0	30	4*)
.13	develop	Add slowly content of 1 sachet sodium carbonate to 250 ml distilled water, use magnetic stirrer. Add 75 µl formaldehyde (37%) to 150 ml developer.	7	9[1]	30	4 (might need adjustment)
.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 fl exible and crystall-free. Should be available in the laboratory

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

Trouble Shooting

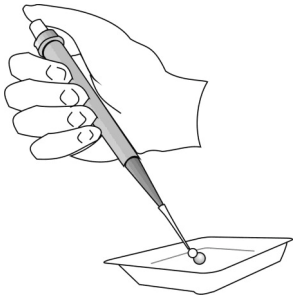
Symptom	Cause	Remedy
After silver staining: No bands or only albumin is visible	Reagents from different sources react differently. Also the water quality may differ.	Silver staining has to be optimized regarding water and chemicals quality in most cases.

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.

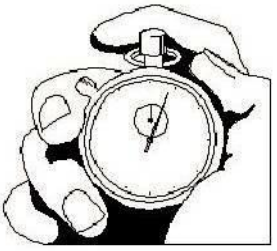
a) If this does not happen, order new chemicals ($AgNO_3$, Na_2CO_3, formaldehyde) and/or check the conductivity of the distilled water.

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 Petri dish 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 the PhastSystem) and enter calculated time into the staining program.



Symptom	Cause	Remedy
Strong, wavy front at the cathode, blurred bands	Salt load of the gel is to high; too much salt in the samples (dilution with 0,9% NaCl), or samples are too high concentrated.	Dilute samples with 0,3 % NaCl or distilled water (see instruction).
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 rehydration pool 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 coo- ling 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.
Lanes have different widths	Sera and CSF have been diluted with wrong NaCl solutions or only with water.	Each lane should have similar loads of salt: ca. 0,3% (w/v) NaCl. Dilute Sera with 0,3% NaCl (w/v); CSF with 0,1% (w/v) NaCl if needed, check instruction.
Bands blurred	Electrodes of the PhastSystem have not been sufficiently cleaned. Take particarly care after SDS electrophoresis!	Carefully clean the electrodes with a tooth brush and distilled water after each run, particularly other SDS electrophoresis (e.g. proteinuria diagnostics). Remove the electrodes from the instrument for this purpose

Results of Isoelectric Focusing