INSTRUCTION MANUAL

SERVALYT™ PRECOTES™ CSF KIT

(Cat. No. 42800.01; Cat. No. 42801.01)

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1. SERVALYT™ PRECOTES™ CSF KIT

1.1. Kit Contents

Kit contains:

5 SERVALYT™ PRECOTES™ CSF
(Gel format**:** 125 x 125 mm/245 x 125 mm, 300 µm)
Anode- and cathode buffer
Electrode wicks
Applicator strips
Manual

1.2. Storage Conditions

The recommended storage temperature is 2 - 8 ℃. If stored at the recommended temperature at least usable until: see expiry date on package.

2. Sample Preparation

2.1. Sample Material

- Cerebrospinal fluid (CSF)
- Serum

2.2. Preparation of the Samples

1. Dilute 10 µl **serum** with 990 µl H_2O.
2. Centifuge CSF and diluted serum for 5 minutes at 14,000 rpm.
3. Apply 10 µl of the respective sample to the pre-run gel.

Note: The **protein concentration** of the **CSF** should be in the range of **40 – 60 mg/l**. If the enclosed protocol for silver staining is used for protein detection, CSF can be applied undiluted. If you want to use less sensitive detection methods (e.g. staining with dyes) the cerebrospinal fluid must be concentrated by ultrafiltation.

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3. Electrophoresis

3.1. Electrophoresis-Short Protocol

Gel formats	245 x 125 mm, 300 µm gel thickness 125 x 125 mm, 300 µm gel thickness
Electrode fluids	
Anode:	Anode fluid 3 (Cat. No. 42984)
Cathode:	Cathode fluid 10 (Cat. No. 42986)
Cooling	Adjust to + 5 °C
Focusing parameters	
Gel format 245 x 125 mm:	2000 V 16 mA 25 W
Gel format 125 x 125 mm:	2000 V 8 mA 16 W
Pre-focusing (without sample)	30 minutes
Sample application	4.0 - 5.0 cm away from the outer edge of the anode
Sample volume	10 µl
Total duration including pre-focusing	3500 Vh or 2.5 hours

3.2. Step by Step Protocol

Parameters in brackets relate to SERVALYT™ PRECOTES™ of 125 x 125 mm format.

1. Pre-cool the cooling plate to 5°C.

2. With a pipette, add 2 ml kerosene (Cat. No. 26940) to the center of the cooling plate (kerosene serves as cooling agent between cooling plate and SERVALYT™ PRECOTES™ gel).

3. Remove the SERVALYT™ PRECOTES™ gel from its carton and cut open the aluthene bag on three sides (two long and one width) using scissors.

Note:
Rubber gloves must be worn when handling acrylamide gels.

4. Role the SERVALYT™ PRECOTES™ gel **with cover sheet** onto the cooling plate (with imprint upwards), avoiding air bubbles. Remove any excess kerosene at margins by means of tissue paper.

5. Take two paper electrode wicks from the pack and lay them on a smooth, clean surface (e.g. glass plate).

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6. Impregnate evenly the electrode wicks.
   2 (1) ml anode fluid 3 (red cap)
   2 (1) ml cathode fluid10 (black cap)

Note:
Impregnated electrode wicks should not be dabbed with blotting paper. The volume of 2 ml relates to the original length of an electrode wick (240 mm).

7. Remove cover sheet with pointed forceps.

8. Place the impregnated electrode wicks on the gel. Anode wick along the **red** line marking, cathode wick along the **black** line marking.

9. Put the **minus** electrode on the cathode wick and the **plus** electrode on the anode wick. Eventually place a heavy glass plate on top.

10. Close the lid of the chamber and connect to power equipment.

11. In the "Set" modus (limitation of parameters) enter the following settings:

    2000 Volt, 16 mA, 25 Watt
    2000 Volt, 8 mA, 16 Watt

12. Isoelectric focusing begins without samples (pre-focusing). **Duration: 30 minutes**

Note:
After switching on, voltage should be between 250 V and 350 V and should rise to 400 V to 600 V after 30 minutes.

13. After 30 minutes, electrophoresis is interrupted and the chamber opened.

14. Position the sample applicatior strip. The sample applicator strip must be placed exactly 4.0 - 5.0 cm away from the outer edge of the SERVALYT™ PRECOTE™ anode.

15. **10 µl** of the respective samples are added to the slots of the applicator strip by means of a pipette (see sample preparation) and electrophoresis is started again.

16. After **3500 Vh** or a **total running time of 2.5 hours**, electrophoresis is completed. The electrode wicks are removed from the gel with forceps and the IEF gel is immediately transferred to 200 ml fixing solution.

Note:
To allow better fixing and any further staining or destaining of the proteins, it is imperative that the* SERVALYT™ PRECOTE™ *is moderately agitated in the respective solutions by means of a shaker. After being used three times, the trichloroacetic acid should be safely disposed of.

17. For silver staining according to Poehling & Neuhoff see paragraph 4, page 6.

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General Note:

If the number of samples does not require a whole gel, the gel may be cut in half with scissors (remove cover sheet before cutting). The remaining gel is then enveloped in the cover sheet and refrigerated at 4℃ in a plastic ba g, as airtight as possible, until required. In this case reduce the electrophoresis parameter amperage and power to half, voltage remains unchanged.

4. Silver Staining

4.1. Reagents and Solutions

- **Fixing solution I**
  150 ml Ethanol (Cat. No. 11094)
  17.3 g Sulfosalicylic acid (Cat. No. 35706)
  285 ml TCA 20 % (Cat. No. 36910)
  fill up to 500 ml H_2O

- **Fixing solution II**
  500 ml Ethanol
  100 ml Acetic acid
  400 ml H_2O

- **Fixing solution III**
  40 ml Glutaraldehyde 25 % (Cat. No. 23115)
  60 ml H_2O

- **Washing solution**
  50 ml Ethanol
  950 ml H_2O

- **Staining solution**
  400 mg Silver nitrate ($AgNO_{3)}$ (Cat. No. 35110)
  1.5 ml Ammonium hydroxide 25 %
  10,5 ml NaOH 0.1 M
  fill up to 100 ml H_2O

- **Developer**
  100 µl Citric acid 5 % (Cat. No. 38640)
  100 µl Formaldehyde solution 37 - 40 %
  fill up to 100 ml H_2O

- **Stop solution**
  10 ml Acetic acid
  90 ml H_2O

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4.2. Procedure

Important points to keep in mind during silver staining

- Use only absolutely clean vessels. The use of vessels soiled with colourants such as Coomassie® etc. may affect results (stainless steel or glass staining vessels are ideal).
- The gel should be kept in constant motion in the respective solution by means of a shaker.
- Ensure that the gel is covered with the respective solution at all times (100 - 200 ml solution depending on the size of the staining vessels are sufficient).
- **Staining solution** and **developer** should be prepared just before they are required.
- You should keep to the following sequence in preparing the staining solution. Dissolve the silver nitrate in 60 ml H_2O. Add 10,5 ml NaOH 0,1 M and mix. Add 1.5 ml ammonium hydroxide 25 % and fill up to 100 ml with H_2O. The staining solution must be as clear as water.

Coomassie® = trademark ICI Ltd.

Staining steps:

1.	Fixing solution I	1 x 20 minutes
2.	Fixing solution II	2 x 10 minutes
3.	Washing solution	2 x 10 minutes
4.	Fixing solution III	1 x 20 minutes
5.	Washing solution	2 x 10 minutes
6.	Rinsing	3 x 10 minutes
7.	Staining solution	1 x 30 minutes
8.	Rinsing	1 x 5 minutes
9.	Developing solution	ca. 2 minutes (vis. inspection)
10.	Stop solution	1 x 5 minutes
11.	Rinsing	2 x 5 min
12.	Air drying at room temperature	

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5. Interpretation

Check protein bands in the cathodic half of the SERVALYT™ PRECOTES™ gel and compare them with those IgG bands which do not appear or show only faint appearance in the serum samples.
These bands are characteristic for IgG synthesis in the brain.

6. Literature

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7. Troubleshooting

Isoelectric Focusing – Staining

Problem	Possible Cause	Countermeasure
No current	Circuit not closed	Remove cables from power supply and check electrophoresis chamber
Low current	Bad contact between electrodes and wicks	Weight electrodes e. g. with a glass plate
Condensation	Too much power	Check settings at power supply and reduce power to 20 W (10 W). Extend focusing time
	Insufficient cooling	Check cooling temperature of the chamber's cooling plate and the connected cooling bath. Remove trapped air bubbles between gel and cooling plate if necessary
	Salt concentration of the sample too high	Desalt sample by dialysis, ultrafiltration or gel filtration
Gel burns through	See "condensation", Gel dries up	Try to stop condensation at the very beginning of the run
Gel burns through at one edge	Electrode wicks longer than gel size	Electrode wicks must not protrude beyond the gel
Distorted bands	Poor soluble or precipitated proteins, high salt concentration in the sample	Spin sample
No band sharpness	Wrong place for sample application	Check place of sample application: 4 - 5 cm away from the outer edge of the anode
Brown strip all over the gel	Electrode solutions contaminated	Always use fresh electrode solutions, aliquote solutions and store seperately until use (- 18 ℃)
No bande in CSF samples	Amount of applied protein to low	Check the IgG concentration of CSF (40 - 60 mg/ml), concentrate if necessary, ideal amount of application: 500 ng IgG
Yellowish or brownish background	Prolonged incubation with silver solution, rinsing time too short	Reduce or increase time, resp.

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