INSTRUCTION MANUAL

SERVALYT™ PRECOTES™
SERVALYT™ PreNets™
Blank PRECOTES™
Blank PreNets™

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1. General considerations

1.1. SERVALYT™ PRECOTES™ and SERVALYT™ PreNets™

SERVALYT™ PRECOTES™ gels are ready to use and designed for horizontal flat bed electrophoresis. The gels consist of thin polyacrylamide layers bound to an inert polyester support film (**GEL-FIX™**) which provides good mechanical stability and easy handling.

In order to prevent the precast gels from drying up and becoming damaged, the surface is covered with a thin film (**GEL-FIX™** for Covers). Each **SERVALYT™ PRECOTES™** gel is packed separately. Refer to the package label for the shelf life of unopened product. Recommended storage temperature is 2 to 8°C (35°F - 46°F).

SERVALYT™ PreNets™ are thin ready-to-use gels prepared from acrylamide poured onto an inert polyester film, similar to **SERVALYT™ PRECOTES™** but with the gel layer not covalently bound to the film. The **SERVALYT™ PreNets™** gel layer can be removed after electrophoresis by lifting it from the film. It can then be transferred to a blotting device to carry out electrotransfer onto a membrane. **SERVALYT™ PreNets™** contain an additional support fabric (polyester net, **NetFix™**) cast into the gel layer which provides mechanical support and stabilizes the gel layer. This makes it very easy to handle the thin gel layer, without risking damage to the gel (tearing, etc.) when transferring it (and your valuable samples!) to a blotter.

The precast **SERVALYT™ PRECOTES™** gels / **SERVALYT™ PreNets™** gels are manufactured according to proprietary expertise of SERVA Electrophoresis GmbH and are inspected by our own quality control.

Each lot is assigned a specific lot number. If you have any questions concerning these products, please contact SERVA Electrophoresis GmbH or your local distributor. Please include the lot number and catalogue number.

Properties / Specifications

- Ready to use, no gel pretreatment required
- High resolution, sharp bands, reproducible results
- Short focusing times
- Fast staining and destaining properties
- Various gel formats, thicknesses and pH fractions available
- Gel can be cut to size
- Safe and gentle handling of SERVALYT™ PreNets™ gels for subsequent blotting
- Easy to dry (in air) for documentation purposes

Polymer concentration	5 % (T) crosslinker (N,N'-methylene-bisacrylamide) 3% (C)
SERVALYT™ concentration	5 %
Support film and thickness	activated polyester film (GEL-FIX™ for PAGE), 180 µm

Cover film and thickness hydrophilic polyester film (GEL-FIX™ for covers), 75 µm

Gel thickness (PAG layer) 0.15 mm or 0.30 mm

Gel formats:

Standard gel, large (**SERVALYT™ PRECOTES™** only)	245 x 125 mm
Standard gel, medium	125 x 125 mm

Package size:

Standard gels, 245 x 125 mm (**SERVALYT™ PRECOTES™** only)	5 pcs.
Standard gels, 125 x 125 mm	5 pcs.

Note: The package contains gels only. Electrode solutions, applicator strips and electrode wicks must be ordered separately. For further information see table below or refer to the main catalogue.

Product name	Package size	Cat. No.	for gel format 245 x 125 mm	for gel format 125 x 125 mm
Electrode wicks,extra long; 300 x 6 x 1 mm	100 pcs.	42972.01	yes	no
Electrode wicks, long 240 x 6 x 1 mm	100 pcs.	42987.03	yes	no
Electrode wicks, standard size, 120 x 6 x 1 mm	100 pcs.	42988.01	no	yes
Anode Fluid 3	50 ml	42984.03	yes	yes
Cathode Fluid 10	50 ml	42986.03	yes	yes

For applicator strips please refer to page 42.

1.2. Blank PRECOTES™ and Blank PreNets™

Blank PRECOTES™ were developed by SERVA to provide a versatile solution to perform isoelectric focusing (IEF) of any pH range. **Blank PRECOTES™** are thin (0.3 mm) polyacrylamide gels onto GEL-FIX™ support film that contain only Bis-Tris buffer pH 6.5. They are given the prefix “blank” to indicate that they are (almost) “empty” gels with a matrix that can be adapted to anything the user wants to be. **Blank PRECOTES™** get equilibrated in the ampholyte mixture of choice (for order information, please see page 36) for only 30 minutes prior to electrophoresis. Resolution is at least as excellent as compared to the resolution obtained with gels of “cast-in” ampholyts.

When blotting is to be performed **Blank PreNets™** are the gels of choice. **PreNets™** gels are prepared with NetFix™ cast into the polyacrylamide matrix adding mechanical stability to the thin gel layer. NetFix™ is an inert polyester net activated to bind polyacrylamid.

After electrophoresis, the gel is lifted easily from its support film (non-binding) and transferred to the membrane of choice, no tearing or damage will occur when handling the thin layer due to the stabilizing effect of the built-in NetFix™. Both methods, semidry and tank transfer, are applicable. Transfer using a Semidry blotter is usually completed within 30 minutes. Handling of **PreNets™** is identical in all steps to operating **PRECOTES™**, including staining and drying.

Benefits are:

- Can be adapted to any pH-range desired by rapid equilibration
- Suited for IEF in the basic pH-range
- Suited for IEF in the presence of urea
- Excellent resolution
- Long shelf life
- Blotting gels available

2. Equilibration of Blank PRECOTES™/PreNets™ with SERVALYT™ Carrier Ampholytes with/without 8 M Urea

2.1. Equilibration solution

	Gel format 125 x 125 mm without Urea	Gel format 125 x 125 mm with Urea	Gel format 245 x 125 mm without Urea	Gel format 245 x 125 mm with Urea
Urea (Cat. No. 24524)	-	12 g	-	24 g
SERVALYT™ (of choice)	2 ml	2 ml	4 ml	4 ml
Glycerol (99%; Cat. No. 23176)	2.5 ml	2.5 ml	5 ml	5 ml
H_2O, dest.	ad 25 ml	ad 25 ml	ad 50 ml	ad 50 ml

Equilibration solution can be used twice. Storage at room temperature for one week is possible.

2.2. Equilibration

- Remove cover sheet (printed) from the gel and incubate it with the gel layer upside down in a tray fitting to the size of the gel. If you use **PreNets™**, remove the support film as well.
- Wash the gel in dest. water for 5 min with gently shaking
- After washing, incubate the gel in equilibration solution for about 30 min

2.3. Surface drying

- After equilibration, rinse the back (GEL-FIX™ support film) of **PRECOTES™** and air-dry the gel at room temperature for max. 30 min
  (Note: Avoid longer drying to prevent urea crystallization!)
- To protect the gel from dehydration, cover it with the cover film (GEL-FIX™ for Covers). Transfer **PreNets™** back on the support film.
- The equilibrated gel is now ready to use for IEF
- Storage of gels without urea: 1 year at 4-8 °C in an airtight packing
- Storage of gels with urea: max. 4 weeks at room temperature in an airtight packing
- Attention: Do not store urea-gels in a refrigerator, because urea will crystallize. After storage longer than 4 weeks the adhesion to the support film and the separation quality of urea-gels will decrease

2.4. Electrophoresis conditions

For example: 125 x 125 x 0.3 mm; electrode distance: 10 cm

	PRECOTES™ / PreNets™ without urea	**PRECOTES™ / PreNets™ with urea**
Temperature	5 °C (41°F)	10 – 15 °C (50 – 59 °F)
Voltage	limited to 2000V	limited to 2000V
Current	limited to ca. 4 mA	limited to ca. 6 mA
Initial voltage	at least 250 - 300 V (adjust with current)	at least 300 - 350 V (adjust with current)
Power	double value of initial current	double value of initial current
Time	ca. 5000 Vh or 3 h	ca. 5000 Vh or 3 h
Example	Set: 2000V, 4 mA, 8 W, 5000 Vh	Set: 2000V, 6 mA, 12 W, 5000 Vh
	Start: 300 V, 4 mA, 1 W	Start: 310 V, 6 mA, 2 W
	Stop: 2000 V, 3 mA, 6 W, 5000 Vh	Stop: 2000 V, 5 mA, 10 W, 5000 Vh

2.5. Order information for SERVALYT™ Carrier Ampholytes

Product	Cat. No.	Size	Product	Cat. No.	Size
SERVALYT™ 2 - 4	42902.01	10 ml	SERVALYT™ 4 - 9	42910.01	10 ml
	42902.02	25 ml		42910.02	25 ml
				42910.03	100 ml
SERVALYT™ 2 - 9	42935.01	10 ml	SERVALYT™ 5 - 6	42924.01	10 ml
Seed-Mix	42935.02	25 ml		42924.02	25 ml
	42935.03	100 ml			
SERVALYT™ 2-11	42900.01	10 ml	SERVALYT™ 5 - 7	42905.01	10 ml
	42900.02	25 ml		42905.02	25 ml
SERVALYT™ 3 - 4	42922.01	10 ml	SERVALYT™ 5 – 7	42936.01	10 ml
	42922.02	25 ml	PGM	42936.02	25 ml
SERVALYT™ 3 - 5	42903.01	10 ml	SERVALYT™ 5 - 8	42949.01	10 ml
	42903.02	25 ml		42949.02	25 ml
SERVALYT™ 3 - 6	42944.01	10 ml	SERVALYT™ 5 - 9	42950.01	10 ml
	42944.02	25 ml		42950.02	25 ml
SERVALYT™ 3-10	42940.01	10 ml	SERVALYT™ 6 - 7	42915.01	10 ml
	42940.02	25 ml		42915.02	25 ml
SERVALYT™3–10	42951.01	10 ml	SERVALYT™ 6 - 8	42906.01	10 ml
Iso-Dalt for 2DGE	42951.02	25 ml		42906.02	25 ml
SERVALYT™ 4 - 5	42923.01	10 ml	SERVALYT™ 6 - 9	42913.01	10 ml
	42923.02	25 ml		42913.02	25 ml
SERVALYT™ 4 - 6	42904.01	10 ml	SERVALYT™ 7 - 9	42907.01	10 ml
	42904.02	25 ml		42907.02	25 ml
SERVALYT™ 4 - 7	42948.01	10 ml	SERVALYT™ 9-11	42909.01	10 ml
	42948.02	25 ml		42909.02	25 ml

For gel format 125 x 125 mm you need 10 ml SERVALYT™ and for 245 x 125 mm 25 ml SERVALYT™.

3. Electrophoresis using SERVALYT™ PRECOTES™/PreNets™

3.1. Sample Preparation

3.1.1. SERVA Liquid Mix IEF Markers 3-10 (Cat. No. 39212.01)

- Apply 5 µl of the ready-to-use marker solution per lane; for silver staining apply 5 µl of a 1:10 dilution per lane.

3.1.2. SERVA IEF Markers 3-10, lyophilized (Cat. No. 39211.01)

- Dissolve 10 mg of protein test mixture in 1 ml demin. H_2O
- Spin sample in a centrifuge at 14,000 rpm for 5 minutes. Load 5 – 10 µl of the supernatant onto the gel

3.1.3. Samples

- Adjust protein concentration according to the number of bands expected:

Staining	Protein concentration
SERVA Violet 17	0.1 – 5.0 mg/ml
SERVA Blau W, Coomassie	0.1 – 5.0 mg/ml
Silberfärbung	0.01 – 0.5 mg/ml

- Use demin. H_2O or 5 % SERVALYT™ solution (according to the pH gradient of the **SERVALYT™PRECOTES™/SERVALYT™PreNets™** gels) to adjust the protein concentration.
- The salt concentration of the samples must not exceed 50 mM. If necessary, desalt the samples by ultrafiltration, dialysis or chromatography.
- If the proteins are difficult to dissolve, add glycerol, urea and/or non-zwitterionic detergents.
- Spin samples for 5 min at 14,000 rpm to remove insoluble protein fragments.
- Sample volume: 5 - 25 µl of the supernatant

3.2. Standard Focusing Program: Summary

Elektrophoresis settings*	Gel Format	Gel Layer [µm]	Voltage [V]	Current* [mA]	Power [W]
	125 x 125 mm	150	2000	3	6
	245 x 125 mm	150	2000	6	12
	125 x 125 mm	300	2000	6	12
	245 x 125 mm	300	2000	12	24

*Initial voltage $\geq$ 200 V

Electrode solutions	:	anode:	anode solution 3	Cat. No. 42984.03
		cathode:	cathode solution 10	Cat. No. 42986.03
Time	:	2.5 h or 3500 Vh	**SERVALYT™ PRECOTES™/ SERVALYT™ PreNets™** gels pH 3 – 10	
		3.5 h or 5000 Vh	all other **SERVALYT™ PRECOTES™/ SERVALYT™ PreNets™** gels	
Temperature	:	5 °C (41°F)		

3.3. Electrophoresis: Step-By-Step Protocol

Setting up SERVALYT™ PRECOTES™ / SERVALYT™ PreNets™ Gels

1 Precool the cooling plate to 5 °C (41 °F).

2 Cut open the **SERVALYT™ PRECOTES™ / SERVALYT™ PreNets™** gel bag along three sides using a pair of scissors (see Figure 1 and 2). For smaller gels, cut to size. Use long-bladed sharp scissors. Store unused portion in original envelope (refold opening).
Note: Always wear gloves when handling polyacrylamide gels.

3 Dispense 0.2 ml kerosene (Cat. No. 26940.01) or Bayol F (Cat. No. 14500.01) evenly in the centre of the cooling plate. The fluid functions as a heat exchange liquid between the cooling plate and the **SERVALYT™ PRECOTES™/SERVALYT™ PreNets™** (see Figure 3).

4 Roll **SERVALYT™ PRECOTES™ / SERVALYT™ PreNets™** gel onto the cooling plate with the sheet face up. Avoid trapping air bubbles between gel and cooling plate (see Figure 4). Position the gel with the red strip (marked on the support film) towards the anode. Remove excess kerosene or Bayol F at the edges of the gel with a tissue paper. A continuous layer of heat exchange liquid must be formed between the

SERVALYT™ PRECOTES™ / SERVALYT™ PreNets™ gel and the cooling plate. Remove trapped air bubbles by lifting and relowering the gel carefully onto the cooling plate.

Electrode Wicks

5 Place two paper electrode wicks (Cat. No. 42988.01) on a clean glass plate (see Figure 5). Make sure that the length of the electrode wicks corresponds to the dimension of the **SERVALYT™ PRECOTES™/ SERVALYT™ PreNets™** gel. If this is not the case, short circuiting or band distortion in the gel may result.

6 Soak electrode wicks evenly with 0.1 ml /cm each of anode fluid 3 (red cap, Cat. No. 42984.03) and cathode fluid 10 (black cap, Cat. No. 42966.03).
Note: Do not blot off remaining electrode solution from soaked wicks using dry paper towels.

7 Carefully peel off cover sheet with a pair of tweezers (see Figure 6).

8 Apply soaked electrode wicks onto the surface of the gel (using a clean pair of tweezers): the anode wick should be placed parallel to the marked red strip, and the cathode wick parallel to the black strip (see Figure 7), the distance between both electrodes should be approximately 10 cm.

Applicator strips

General consideration for using applicator strips:

Applicator strips can be used for more than one separation. Rinse the strips with an appropriate volume of demineralized water and dry them. It is important that the applicator strip is absolutely clean and free of dust particles. Dust particles prevent proper contact of the applicator strip to the gel. This will interfere with sample loading and may cause band smearing and distortion in the pherogram.

Select the Correct Applicator Strip

The size of the applicator strip is selected according to the sample volume. Strips are available in different sizes (see table below):

Product (material)	Cat. No./ pcs. per package	Sample volume [µl]	max. sample number	Total size [mm]	Application
Applicator strips 7 x 1 mm (silicon strip)	42989.01 (3 pcs.)	10	24	strips 260 x 6 x 1[1]	for gels which are 245 mm wide, samples cont. 10 - 30 mg/ml protein
Applicator strips 3.5 x 2 mm (silicon strip)	42915.01 (6 pcs.)	5 - 10	15	strips 100 x 6 x 1[1]	for gels which are 125 mm wide, samples cont. 0.5 – 1 mg/ml protein
Applicator strips 2 x 3.5 mm (silicon strip)	42914.01 (6 pcs.)	5 - 10	19	strips 100 x 6 x 1[1]	for gels which are 125 mm wide, samples of low protein concentration (0.5 – 1 mg/ml)
Applicator strips 3.5 x 2 mm (silicon strip)	42899.01 (3 pcs.)	5 - 10	43	strips 240 x 6 x 1[1]	for gels which are 245 mm wide, samples samples of low protein concentration (0.5 – 1 mg/ml)
Applicator strips kit (silicon strips))	42937.02 (4x 1 pcs.)				contains one strip each of Cat. No. 42989, 42915, 42914, 42899
Applicator pieces (paper)	42880.01 (200 pcs.)	15 - 25		one piece 10 x 5[1]	for special applications such as Hp,PGM, etc.

[1] Length x width x depth

Position Applicator Strip Properly:

- Parallel to wicks, across entire width of gel.
- Place at the center of the gel (midway between electrodes) if the pI values of the proteins contained in the sample are unknown.
- Shift position of strip towards anode if the sample contains mainly basic proteins.
- Shift position of strip towards cathode if the sample contains mainly acidic proteins.
- Not within 25 mm away from the wick.

Sample Application Pieces (paper):

Apply sample application pieces with their longer side parallel to the electrodes. The application pieces may be placed in any order at various positions or aligned with the gel.

Sample Loading

9 Positioning of the applicator strip and sample loading (see Figure 8):
Position applicator strip at one end of the gel and roll strip into position with a downward pressing motion. The applicator strip must not extend beyond the edge of the **SERVALYT™ PRECOTES™ / SERVALYT™ PreNets™** gel and must be **immaculately clean**. Any adhering dust particles or lint will prevent proper contact with the gel. The result may be seepage of sample fluid and consequently smearing of patterns. If the strip does not adhere properly, remove it, wash it in distilled water and dry it. Avoid detergents which make samples creep under the strip. As control, apply 5 µl of SERVA Liquid Mix IEF Markers 3-10 (Cat. No. 39212.01) or 10 µl of SERVA protein test mixture for pI determination pH 3-10 (Cat. No. 39211.01; a final concentration of 10 mg protein / ml is recommended).

Positioning the Electrodes

10 Center **cathode** on top of the cathode wick.
Center **anode** on top of the anode wick (see Figure 9).

11 It is important that the electrodes have even and proper contact with the wicks. Insufficient contact of the electrodes and wicks will cause distortion of bands (wavy). By positioning a weight (glass plate, min. 300 g) across both electrodes, sufficient pressure can be applied, providing proper contact between the electrode and the wicks.

12 Close the lid of the chamber and start the electrophoresis.

13 Choose the settings for the power supply from the following table according to the gel format and thickness:

Size	Gel layer thickness [µm]	Voltage [V]	Current* [mA]	Power [W]
125 x 125 mm	150	2000	3	6
245 x 125 mm	150	2000	6	12
125 x 125 mm	300	2000	6	12
245 x 125 mm	300	2000	12	24

*Initial voltage ≥ 200 V

14 Start the electrophoresis

15 Stop the electrophoresis after 3500 to 5000 Vh or 2.5 to 3.5 hours. Switch off the power supply and remove the lid of the electrophoresis unit. Carefully remove the wicks and the applicator strip. Transfer the **SERVALYT™ PRECOTES™** gel into a tray containing the fixative solution (see Detection, page 43). If blotting **SERVALYT™ PreNets™**, continue with chapter 5 (see Blotting, page 47).

Note: Good results for protein separation are obtained under the standard conditions listed above. Electrophoresis separation conditions can be adapted to your needs by increasing the current in the early phase of electrophoresis (resulting in higher voltage values) which reduces separation time. It is important that the power applied to the gel do not exceed the indicated ratings due to the capacity of the cooling plate. In some applications, increase in the final voltage up to a value of 2500 V (max.) may improve band sharpness. If proteins with a molecular weight above 150,000 Da are present in the sample, increase the electrophoresis temperature up to 10 °C (50 °F).

If prefocusing is necessary (depending on the properties of the loaded samples), perform electrophoresis for 20 minutes without samples. Stop the electrophoresis run, position the applicator strips on the gel, and load the samples. Continue the electrophoresis run. The applicator strip remains on the gel during the run, but sample application pieces must be removed from the gel after another 45 minutes. They can be removed using a pair of tweezers after electrophoresis has been stopped and the electrode lid has been removed. Reposition the electrodes and continue the run.

4. Detection

4.1. Protein Staining : SERVA Violet 17

4.1.1. Reagents and Solutions

Fixative Solution	20% (w/v) trichloroacetic acid (Cat. No. 36913.01)
SERVA Violet 17 Stock Solution 1*	Dissolve 500 mg SERVA Violet 17 powder (Cat. No.35072.01) or 20 tablets of SERVA Violet 17 (Cat. No. 35075.01) in 250 ml demin. H_2O
Stock Solution 2	20% (w/v) phosphoric acid: 70 ml of 85% phosphoric acid add demin. H_2O to make 500 ml.
Destaining Solution	3% (w/v) phosphoric acid: 20 ml of 85% phosphoric acid add demin. H_2O to make 1,000 ml.

The colloidal Violet 17 staining solution is stable for 20 minutes, only. It is not reusable.

4.1.2. Procedure

1 Fixation Carefully remove electrode wicks after electrophoresis with a pair of tweezers. Transfer gel immediately into a tray containing 200 ml 20 % trichloroacetic acid. Incubate gel in fixation solution.
Time: 20 minutes

Note: The use of a shaker is recommended in all steps of detection. Gentle motion (50-100 rpm) of the gel in the solution will help to optimize fixation, staining, and destaining steps.
Always wear appropriate clothing and gloves when working with trichloroacetic acid.

2 Rinsing Rinse the gel in 200 ml destaining solution.
Time: 1 minute

3 Staining Prior to use, mix 100 ml of stock solution 1 and 100 ml of stock solution 2 and incubate the gel.
Time: 10 minutes

4 Destaining Destain the **SERVALYT™ PRECOTES™** gel in 200 ml destaining solution until background is clear.
Time: 2-3 x 10 minutes

5 Rinsing Incubate the **SERVALYT™ PRECOTES™** gel in 1 – 2 % (w/v) glycerol.
Time: 2 x 5 - 10 minutes

6 Drying Dry **SERVALYT™ PRECOTES™** gel in a jet of warm air or at room temperature.

4.2. Protein Staining : SERVA Blue W

4.2.1. Reagents and Solutions

Fixative Solution	20 % (w/v) trichloroacetic acid (Cat. No. 36913.01)
Staining Solution	Dissolve 100 mg SERVA Blue W powder (Cat. No. 35053.02) or 4 SERVA Blue W tablets (Cat. No. 35053.01) in 250 ml demin. H_2O (the solution is stable for several months at room temperature).
Destaining Solution	0.4 % (w/v) trichloroacetic acid: 20 ml of 20 % (w/v) trichloroacetic acid (Cat. No. 36913.01) add demin. H_2O to make 1,000 ml.

4.2.2. Procedure

1 Fixation Carefully remove electrode wicks after electrophoresis with a pair of tweezers. Transfer gel immediately into a tray containing 200 ml 20 % trichloroacetic acid. Incubate gel in fixation solution.
Time: 20 minutes

2 Neutralization Rinse the gel in 200 ml demin. H_2O.
Time: 1 minute

3 Staining Prior to use, add 5 ml of fixation solution to 250 ml staining solution and incubate the gel.
Time: 10 minutes

4 Destaining Incubate the gel in destaining solution (200 ml) until the background is clear.
Time: 2-3 x 10 minutes

5 Rinsing Incubate the **SERVALYT™ PRECOTES™** gel in 1 – 2 % (w/v) glycerol. Time: 2 x 5 - 10 minutes

6 Drying Dry the **SERVALYT™ PRECOTES™** gel in a jet of warm air or at room temperature.

Note: The use of a shaker is recommended in all steps of detection. Gentle motion (50-100 rpm) of the gel in the solution will help to optimize fixation, staining, and destaining steps.
Always wear appropriate clothing and gloves when working with trichloroacetic acid.

4.3. Silver Staining

Optimized for **SERVALYT™ PRECOTES™** gels (layer thickness: 0.15-0.3 mm)

4.3.1. Reagents and Solutions

Fixative Solution I	20 % (w/v) tichloroacetic acid (Cat. No. 36913.01)
Fixative Solution II	80 ml glutaraldehyde (25 %) (Cat. No. 23115.01) add demin. H_2O to make 1,000 ml
Rinsing Solution	300 ml ethanol (96 %) (Cat. No. 11094.01) add demin. H_2O to make 1,000 ml
Preequilibration Solution	40 mg $Na_2S_2O_3$ add demin. H_2O to make 200 ml
Staining Solution	200 mg $AgNO_3$ (Cat. No. 35110.01) 75 µl formaldehyde (37 %) add demin. H_2O to make 100 ml.
Staining Solution	6 g Na_2CO_3 (Cat. No. 30181.01) 10 mg $Na_2S_2O_3$ 75 µl formaldehyd (37 %) add demin. H_2O to make 100 ml.
Stop Solution	10% (v/v) acetic acid

4.3.2. Procedure

1 Fixation	Incubate the gel in fixative solution I. Time: 20 minutes
2 Rinsing	Treat the gel with rinsing solution. Time: 10 minutes
3 Fixation	Soak the gel in fixative solution II. Time: 10 minutes
4 Rinsing	Rinse the gel twice with rinsing solution. Time: 2 x 10 minutes
5 Preincubation	Incubate the gel in preequilibration solution. Time: 1 minute
6 Rinsing	Rinse the SERVALYT™ PRECOTES™ gel with demin. H_2O. Time: 3 x 20 seconds
7 Staining	Incubate the gel in staining solution. Time: 20 minutes

8 Rinsing	Rinse the SERVALYT™ PRECOTES™ gel with demin. H_2O. Time: 2 x 20 seconds
9 Detection	Incubate the gel in developing solution until bands become visible.
10 Stopping	Treat gel with stop solution. Time: 5 minutes
11 Rinsing	Incubate the **SERVALYT™ PRECOTES™** gel in 1 – 2 % (w/v) glycerol. Time: 2 x 5 - 10 minutes
12 Drying	Dry the **SERVALYT™ PRECOTES™** gel in a jet of warm air or at room temperature.

4.3.3. Important Note for Silver Staining

- Please be sure to use clean tubs for the gel staining procedure. Traces of remaining dyes like Coomassie Blue etc. may interfere with your detection procedure. For best results use staining tubs made from stainless steel or glass.
- The use of a shaker for all subsequent detection steps is recommended (50 -100 rpm).
- Please ensure that the gel is always covered by a sufficient amount of solution. Usually, a solution volume of 100 – 200 ml is sufficient for many common staining trays.
- Prepare the staining solution and the developing solution immediately before use.

5. Blotting with SERVALYT™ PreNets™

After electrophoresis, perform blotting with **SERVALYT™ PreNets™** gels. Any transfer method is acceptable.

5.1. Tankblotting

1 x Towbin Buffer for Western Blotting (Cat. No. 42558.01)	192 mM Glycin 25 mM Tris 20 % (v/v) Methanol 0.05 – 0.1 % (w/v; optional) SDS

5.2. Semidry Blotting

5.2.1. Continuous:	0.5 x Laemmli Buffer (Cat. No. 42556.01)	
5.2.2. Discontinuous:	Semi-dry Buffer Kit for Western Blotting (Cat. No. 42559.01)	
	consits of:	
	Anode buffer I:	0.3 M Tris, 20 % methanol
	Anode buffer II:	0.03 M Tris, 20 % methanol
	Cathode buffer III:	0.025 M Tris/HCl (pH 9,4) 0.04 M 6-aminocapronic acid 20 % (v/v) methanol

For electrophoresis parameters and time settings, please refer to the manufacturer`s manual of your electrophoresis tank. To control the efficiency of your protein transfer, stain membrane (e.g. Ponceau S, Cat. No. 33427.01) and gel (see Chapters 3.1. and 3.2.) simultaneously after blotting.

6. Troubleshooting

6.1 Electrophoresis

Problem	Probable cause	Remedy
No current	Circuit not closed	Remove cables from power supply and check electrophoresis chamber
Low current	Poor contact between electrodes and wicks	Place weight on top of electrodes; e.g. 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 temperature settings of the cooling plate chamber and the connected cooling bath. Air bubbles may be trapped between gel and cooling plate. Lift gel to remove.
	Salt concentration of sample too high	Desalt sample by dialysis, ultrafiltration or gel filtration.
	Electrode solution or wicks reversed	Check polarity.
Gel burns out	See „Condensation“, Gel dries up	Try to stop condensation at the very beginning of the run
	Gel overloaded	Reduce protein concentration or sample volume. Dilute sample if necessary.
Gel burns out at one edge	Electrode wicks longer than gel size	Electrode wicks must not protrude beyond edge of gel.

6.2 Staining

Problem	Probable cause	Remedy
Gel does not stick to support	Excessive incubation in TCA	Store gel in (m)ethanol (50 %) / glacial acetic acid (10 %) if necessary
Gel breaks when drying		Incubation in 1 - 2 % (w/v) glycerol (2x 5 – 10 min) before drying.
SERVA Violet 17		
Blue background	SERVALYT™ not removed completely	Prolong incubation time in TCA, use TCA solution up to three times
Blue precipitation on the gel	TCA precipitates dye	Rinse gel thoroughly with water after fixing
Green background	Concentration of dye too low	Prepare a new stock solution
Green background; greenish bands that fade during drying	pH value in the gel too low after destaining procedure, gel was not neutralized properly	Neutralize by rinsing the gel with water or 1% glycerol. Restain the gel.
SERVA Blue W, Coomassie		
Blue background	SERVALYT™ not removed completely	Prolong incubation time in TCA, use TCA solution up to three times
	TCA precipitates dye	Rinse gel thoroughly with water after fixing
	Staining solution too old	Prepare a new staining solution
Silver Staining		
Brown strip across the gel	Electrode solutions contaminated	Always use fresh electrode solutions. Aliquote and store the solution separately until use
yellowish or brownish background	Prolonged incubation in silver solution, rinsing time too short	Reduce time of rinsing steps
No bands	Rinsing steps too long, silver ions washed away	Reduce time of rinsing steps

6.3 Separation

Problem	Probable cause	Remedy
pH gradient distorted	Focusing time too long	Reduce focusing time
	pK value is temperature dependent	Adjust to recommended temperature
Wavy pH lines		
a) without sample	Uneven contact between electrodes and the gel	Place weight on top of electrodes, e.g. glass plate
	Electrode wicks too wet or unevenly soaked in electrode buffers	Use 0.1 ml electrode solution per cm of wick
	Salt concentration in electrode solutions too high	Use recommended solutions
b) with sample	Protein concentration in the sample too high, gel overloaded	Reduce protein concentration or sample volume. Dilute sample if necessary
	Protein concentration varies from lane to lane	Apply equal sample volumes/amounts of protein or apply samples in ascending order
	Applied salt concentration varies from lane to lane	Dissolve protein in buffer with the same ionic strength or the same buffer if possible
	Electric field too high	Apply sample at max 600V. Reduce current if necessary
Distorted bands	Sample contains poorly soluble or precipitated proteins or salts	Spin sample
	Gel overloaded	See above
	Lipid concentration in the sample too high	Reduce lipid concentration by ultracentrifugation. Add zwitter- or nonionic detergents

Problem	Probable cause	Remedy
	Applicator strips and applicator pieces cause retardation of proteins	Remove applicator strips or applicator pieces 30-45 min after sample application
	Sample too old, denatured	Always prepare sample just before electrophoretic separation. Avoid freeze and thaw cycles.
	High MW proteins do not reach pI	Extent focusing time or use gel with large pore size, e.g. agarose.
No band sharpness	Focusing time too short	Extend focusing time
	Final voltage at the end of the run not reached	Check settings at power supply; salt concentration in the sample may be too high, final voltage at the end of the run is not reached
	Wrong sample application site	Evaluate optimum position for sample application. Position applicator strip diagonally for testing.
	Fixation procedure insufficient	Check fixation procedure
Missing bands	High molecular weight proteins	Use agarose gel matrix
	Precipitations of proteins at application site	Check different materials for sample application; vary/evaluate test position for sample application, prefocus gel before sample application
	Electric field too high	Apply sample at max 600V. Reduce current if necessary
	Detection method used not sensitive enough	Increase sample volume, use alternative protein staining/detection methods, e.g. silver staining or immunoblotting

Problem	Probable cause	Remedy
Protein focussing results in more than one band	Different binding characteristics of cofactors and ligands	Saturate protein completely ensure correct position of application
	Various oxidation forms	Check sample preparation e.g. add DTT
	Different conformational structures (oligomeres or subunits)	Perform electrophoresis under denaturing conditions using > 7 M urea
	Different levels of glycosylation phosphorylation, acetylation, etc. of proteins	Change sample preparation, e.g. neuraminidase treatment
	Proteolytic degradation of proteins	Check sample preparation, e.g. add protease inhibitors

Get Started with Isoelectric Focusing using SERVALYT™ PRECOTES™

Abb. / Figure 1

- Alles was für die IEF benötigt wird
- All what you need to run IEF

Abb. / Figure 2

- Beutel an drei Seiten aufschneiden
- Cut open bag along 3 sides

Abb. / Figure 3

- Etwas Kerosin auf die Kühlplatte geben
- Add kerosine to the cooling plate

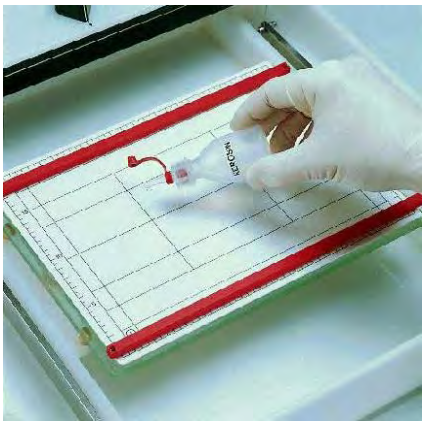
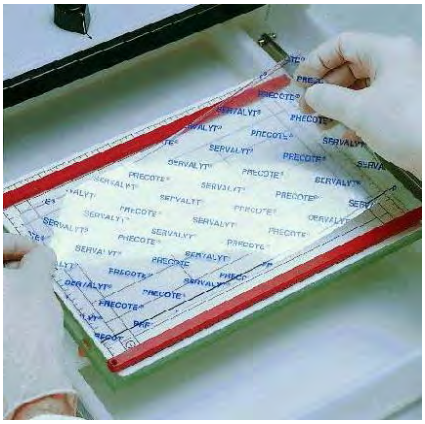


Abb. / Figure 4

- Gel auf die Kühlplatte auflegen
- Position gel on cooling plate



Get Started with Isoelectric Focusing using SERVALYT™ PRECOTES™

Abb. / Figure 5

- Dochte mit Elektrodenpufferlösung tränken (feucht, nicht naß)

- Apply electrode buffers to wicks (moist, not wet)

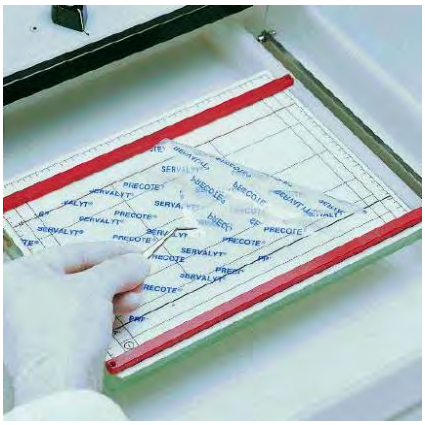


Abb. / Figure 6

- Deckfolie entfernen
- Peel off covers

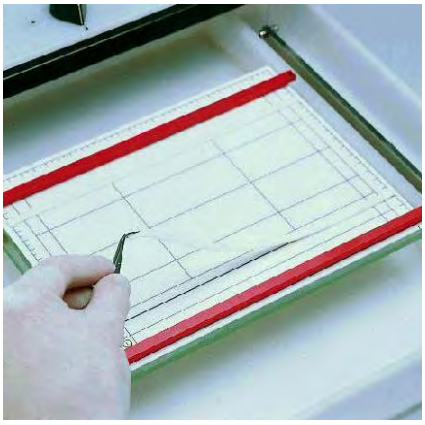


Abb. / Figure 7

- Befeuchtete Elektroden auf die Gelkanten auflegen

- Position buffer-soaked wicks at ends

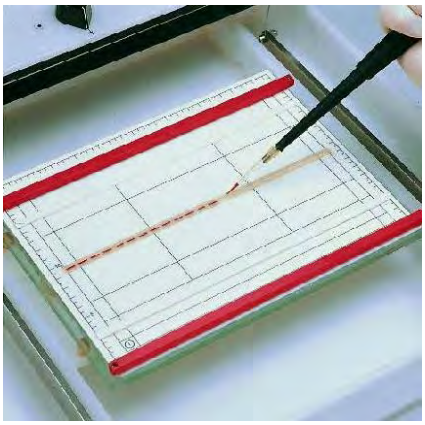


Abb. / Figure 8

- Applikatorstreifen auflegen
- Use an applicator strip and load samples

Abb. / Figure 9

- Elektroden auf die Dochte auflegen
- Place an electrode on top of wicks

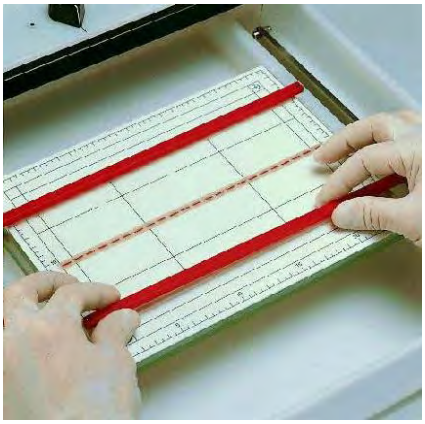


Abb. / Figure 10

- Eventuell eine Glasplatte auf die Elektroden legen und Elektrophorese starten

- Eventually put a heavy glass plate on top and start electrophoresis at given conditions

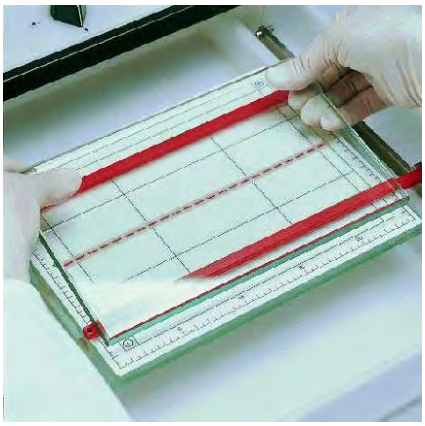


Abb. / Figure 11

- Färben nach beendeter IEF: siehe Text (4.1. ff)
- Stain upon completion of the IEF (see 4.1.)