Instruction Manual: 1D Electrophoresis with SERVA SDS Urine Gel Kit 25 S

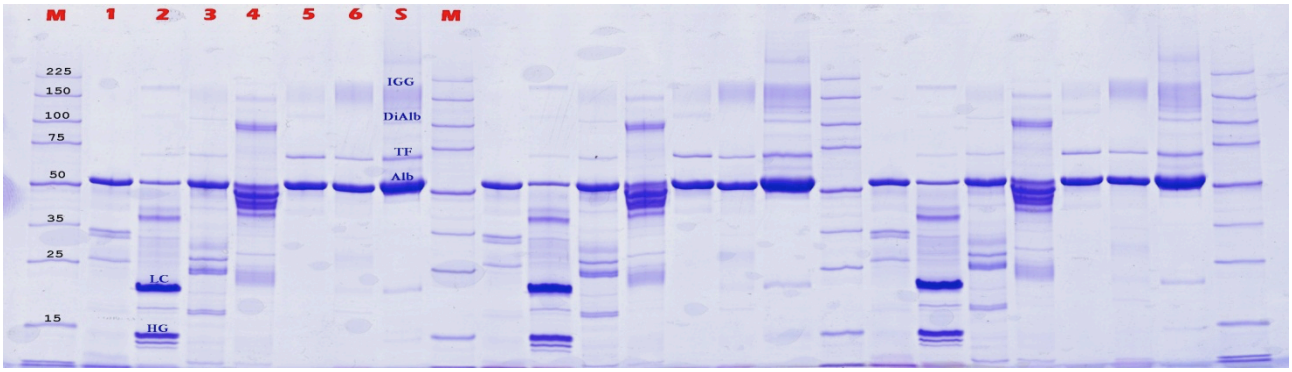

Fig. 1: Urinary proteins on a SDSGel 10% 25S (Samples: Dr. Chr. Weber, Krankenhaus Reinkenheide, Bremerhaven, Germany), M: Marker, S: Serum, 1,3: tubular proteins, 2: Bence Jones proteins, 4: nonselective glomerular and tubular proteins, 5,6: glomerular proteins. Coomassie staining

The SDS Urine Gel Kit 25 S contains ready-to-use SDS polyacrylamide gels, running buffers, electrode wicks, and a 10x conc. sample diluter. The gels are polymerized on plastic backing, have a size of 25 x 12.5 cm x 0.45 mm thickness and contain 25 x 15 µL sample slots (25S). For long shelf-life and optimal separation conditions a Tris-tricine gel chemistry is applied, using a gel buffer with a pH value below 7.

Ordering information:

SDS Urine gel kit 25 S 4 gels incl. 10 x conc. sample buffer, electrode buffers, and wicks	Cat. No. 43391.01
Cooling fluid 50 mL	Cat. No. 43371.01
SERVA HPE™ Coomassie Staining Kit	Cat. No. 43396.01

Sample Concentration

The protein concentration of the samples for an electrophoretic separation must be within a certain range. If the protein content is too low, proteins cannot be detected, if it is too high, overloading effects cause blurred band patterns. Physiological urines have a low protein concentration and only a weak albumin band should be detectable. The protein concentration range depends on the sensitivity of the visualization process:

Coomassie Blue Staining:
This staining is not very sensitive but allows quantification..
Concentration range per slot with 15 µL sample volume: 3 µg (200 mg/L = 20 mg/dL)

Silver Staining:
Very sensitive, but quantification is not reliable.
Concentration per slot with 15 µL sample volume: 0.3 µg (20 mg/L= 2 mg/dL)

Sample Buffer „10x conc“:

15 mL Sample Buffer 10x is supplied with this kit. Simply add 10% (v/v) of this „10 x conc“ buffer to the sample (tab. 1). In this way the samples will be only slightly diluted. This sample buffer is non-reducing!

Note for high protein samples: Do not load more than 5 µg per slot. If necessary, the samples can be diluted with H_2O dist. to prevent overloading effects. Then add 10% of the 10x sample buffer to the sample volume. Do not reduce the urinary SDS-proteins.

Tab. 1: Dilution of urinary samples for Coomassie Blue staining

Protein Concentration	Test - Stick	Urine	H_2O_{bidest}	10x Sample Buffer
negative		90 µL	—–	10 µL
0.3 g/L= 30 mg/dL		45 µL	45 µL	10 µL
1 g/L = 100 mg/dL		20 µL	70 µL	10 µL
5 g/L = 500 mg/dL		5 µL	85 µL	10 µL

Do not reduce the Samples

Reducing with 2-mercaptoethanol or dithiothreitol will dissolve the quaternary structures of the Immunoglobulins. These molecules show a native molecular weight of 160 kDa, after reducing only heavy (43 kDa) and light (20 kDa) chains will appear. Urinary proteins should be separated with intact quaternary structures. Please be aware: Because the disulfide bridges are opened up, the polypeptides cannot not completely unfold : thus the separation distances of these SDS-protein-micelles are not exactly proportional to their molecular weights! To identify the bands: Run known serum-protein samples (Albumin, IgG, ...) to compare them to the urinary proteins.

Heating to 95 °C for 3 minutes

To coat the proteins with the SDS-molecules the sample-SDS-mixtures should be heated to 95 °C for 3 minutes. The reagent cups are placed into a reagent cup heater. This procedure leads to a uniform negative charging of the proteins and the molecular weights can be estimated after the electrophoresis run.

Important: Only use the SERVA buffer kit for the running buffers.

1. Lay two electrode wicks into the compartments of the PaperPool. Apply 45 mL of the respective electrode buffer to each electrode wick. (Fig. 2). Distribute the solution evenly with a roller.

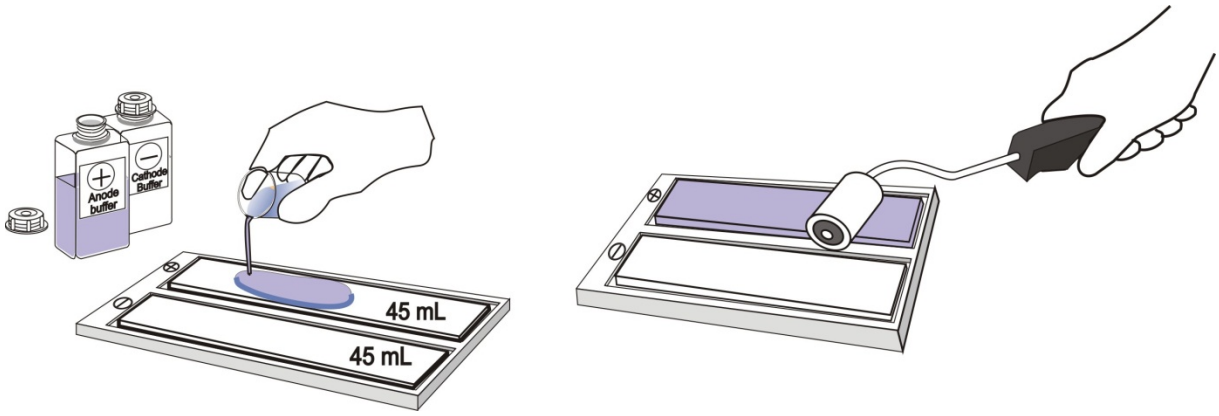

Fig. 2: Soaking the electrode wicks and rolling them for an even distribution of the buffer.

2. Apply 3 mL cooling contact fluid onto the cooling plate.
3. Remove the gel from its packaging. Remove the cover-film. Grip the gel (surface up) at the two lateral edges at the protruding film, bend it like an "*U*" and slide the film-backing left and right on the cooling plate to distribute the cool contact fluid evenly (Fig. 3). Remove excess cooling fluid along film edges with lint-free tissue paper.

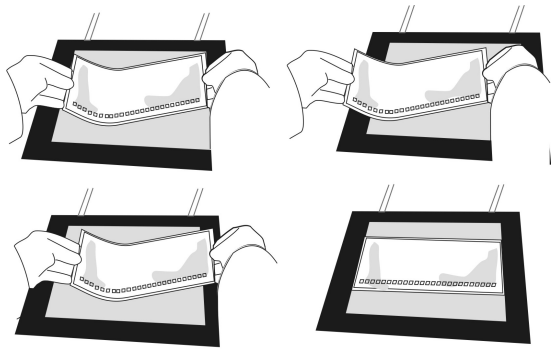

Fig. 3: Applying the gel on the cooling plate.

4. Remove excess electrode buffer from the wicks by tilting the electrode wicks along one long edge and dab it on the PaperPool bottom (fig. 4).

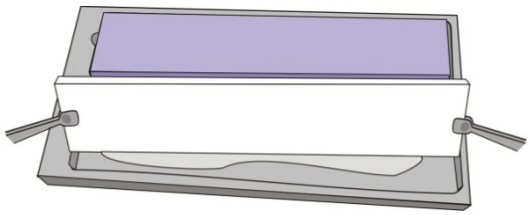

Fig. 4: Dabbing the electrode wick on the PaperPool bottom to remove excess buffer.

5. Place the electrode wicks onto the gel edges overlapping them by at least 2 mm. Hold wicks horizontally! Never slanted, because this would cause unequal buffer concentration along the wick. Smooth out air bubbles with bent tip forceps.
6. Pipette 15 µL of each sample into the sample wells.
7. Clean platinum electrode wires before (and after) each electrophoresis run with moist tissue paper.
8. Close the lid while lowering the electrodes on the wicks, plug the cables in, switch on the cooling (15 °C).

10. Turn on your power supply and start the run according to table 2.

Tab. 2: Running conditions (15°C): total 2 h

1 Gel:	Limit V	Limit mA	**Set W**	**Time**
phase 1	600 V	42 mA	30 W	1 h
phase 2	1000 V	50 mA	60 W	1 h

Protein Staining

The best results are obtained with Coomassie Bue Colloidal Staining:
SERVA HPE™ Coomassie Staining Kit (Cat. No. 43396.01)

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