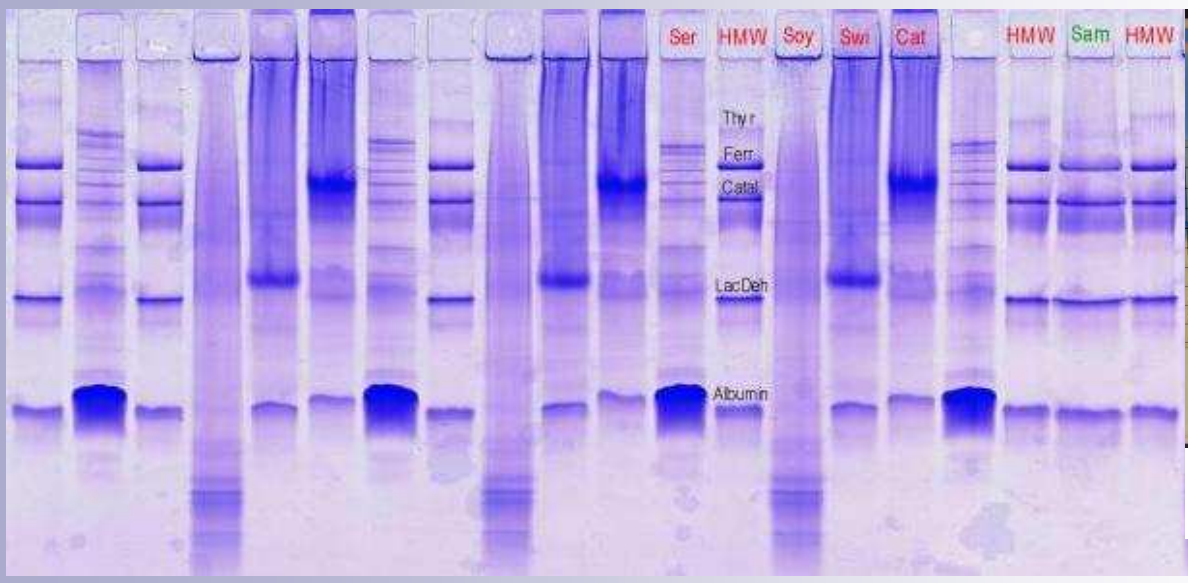
Native Anodal Protein Electrophoresis with the Protein Buffer Kit Anodic

EDC V3 9/14



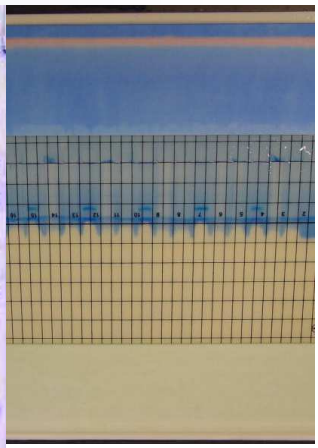


Fig.1b: *Blue Native electrophoresis*

Fig.1a: *anodic native electrophoresis using DryGel Elpho 12.5% and the Protein Buffer Kit Anodic*

General:
For native anodal protein separations most frequently a discontinuous buffer from Ornstein and Davies [1] is applied. For some applications, a very high resolving power for proteins is needed, this can be achieved by this Buffer Kit. This buffer kit contains a buffer system, which produces extremely sharp bands, resulting in a very high resolution even within short separation distances of 8 cm. The rehydration buffer contains a specific amphoteric compound and has a pH-value of 7.3.
Due to this neutral pH value Blue Native electrophoresis [2] is possible (fig.1b) and will be described below.
The DryGel 12.5% 24S is recommended as electrophoretic gel. See figure 1a

Protein Buffer Kit Anodic consists of:
Rehydration buffer,**Cathode buffer**, **Anode buffer, Sample buffer** :
4 Drying Cardboards
Electrode strips: 8 electrode strips 5.0 x 25.3 cm.
Additional needed: **Urea** (Merck 8488)

The following equipment is recommended:

Electrophoretic Hardware:	
flatbed professional	(edc-prof2836)
Multiphor	(GE-18-1018-06)
Additionally necessary:	
DryPool Combi	(edc-ME-D)
Tray for rehydration of dry gels (normal size) and soaking electrode strips	
Steel Tray Small for hot Coomassie staining	(edc-WM-N1)

Sample buffer (also for Blue Native electrophoresis):
5 mg Dodecylmaltoside, 25 µl 1 % Triton X100 and 80 µl Bromophenolblue (1%) should be added to the 100 µl Rehydration buffer. Up to 6M Urea can be added if necessary.

Rehydration of the DryGels

Rehydration solution: 3 g urea (if needed) made up to 50 ml with rehydration buffer.
Lay the DryPool Combi onto a horizontal table, fill the 50 ml solution into the chamber. Lay the edge of the gel-support - with the gel surface facing down - into the rehydration buffer (fig. 2) and slowly lower it, avoiding air bubbles. Using foreceps, lift the gel up to its middle, and lower it again without catching air bubbles, in order to achieve an even distribution of the liquid (fig. 3).
2 hours later the gel has reswollen completely and can be removed from the DryPool. Wipe away any urea crystals from the rear surface. Remove buffer completely from the gel surface and the sample wells using on of the drying cardboards.

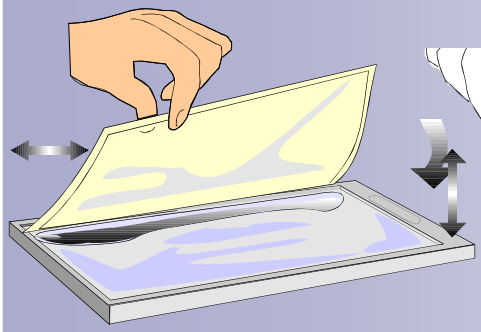


Fig2: Placing the DryGel into the DryPool Combi

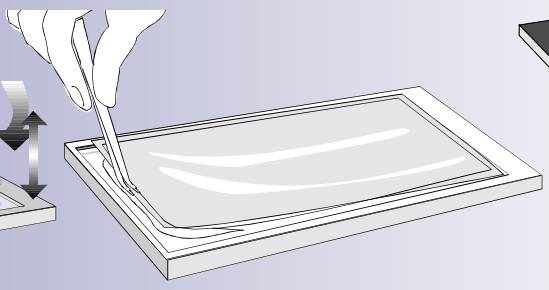
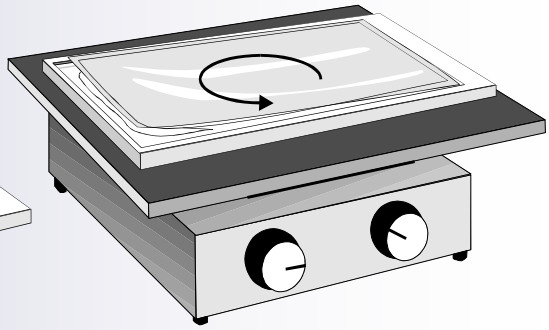


Fig 3: Lifting the gel for an even distribution of the liquid…..



….then use a rocking platform

Application of the Gel and the Electrode Strips

Switch on the thermostatic circulator, adjusted to 10 °C. Spread 1.5 ml of kerosene onto the cooling plate of the electrophoresis chamber, in order to ensure good cooling contact. Place the gel (surface up) onto the center of the cooling plate: The side containing the sample wells is orientated towards the cathode (fig. 6; Multiphor II: cathodal side of the wells matching with line no. 4).
Lay two of the electrode wicks into the compartments of the DryPool, the convex side orientated to the top. Apply 25 ml of the anode and cathode buffers respectively to the strips (fig. 4). The anode buffer contains a green dye to avoid mistakes (this does not spread evenly in the wick).
Blue Nativ Electrophoresis: add 4 ml Coomassie G250 (0.1% , in Ethanol) to the 25 ml cathode buffer. The run should look like in figure 1b.

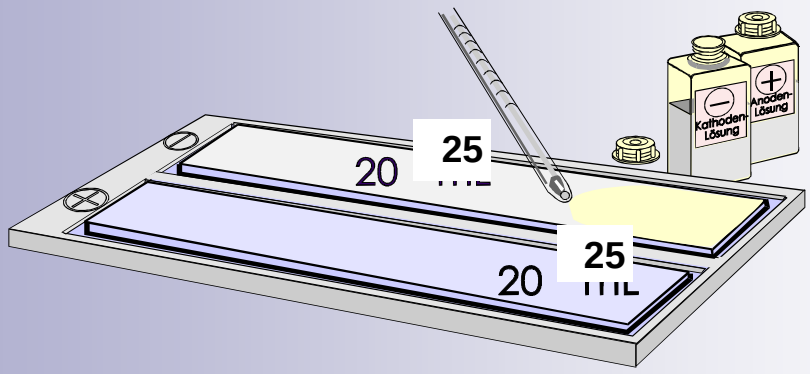


Fig. 4: Soaking the electrode strips with the anode and cathode buffer in

Standards

1. IEF-standards (3-10 or 7-10) can be used.
2. Protein Mix: (je 5 µg / ml): Amyloglucosidase - pI 3.5*, Glucose oxidase - pI 4.2*, Trypsin inhibitor - pI 4.5*, β-Lactoglobulin - pI 5.15 / 5.3*, Carboanhydrase - pI 6.0, Myoglobin horse - pI 6.9 / 7.35 (300 ng in 12,5 µl), Lentil lectin - pI 7.75 / 8.0 / 8.3, Ribonuclease A - pI 9.45, Cytochrome C - pI 10.65.
   Dilution Coomassie: 50+150, pipet 12.5 µl
   * not necessary

Place the cathode strip onto the cathodal edge, matching the grid's lines. Place the anode strip onto the anodal edge of the gel in the same way.
See fig. 5 and 6. Always apply cathode wick first (more sensitive than the anode!).
Smooth out air bubbles by sliding bent tip foreceps along the edges of the wicks laying in contact with the gel (first cathode, then anode!).

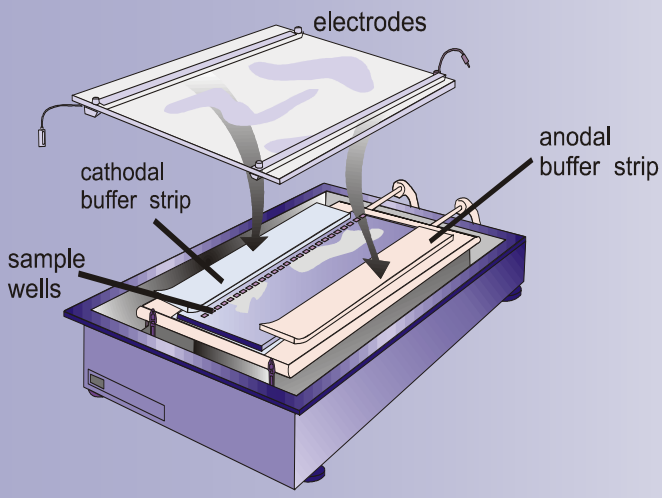


Fig 5: Horizontal electrophoresis apparatus: GE's „Multiphor"

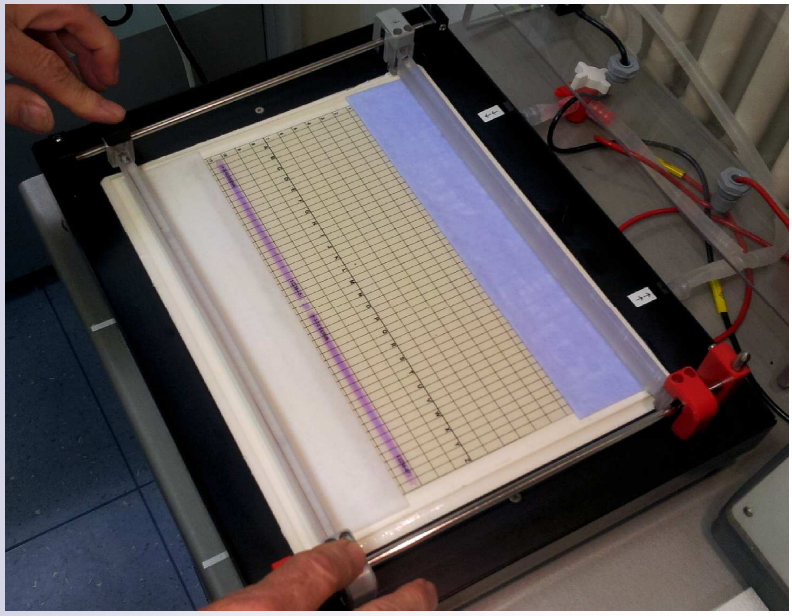


Fig 6: Arrangement of gel, buffer strips and electrodes. Behind the samples is the cathode!

Sample application and electrophoresis
Apply 5 µl of each sample tube to each well using a micropipette (or use appropriate multipipette, microtiter plate standard distances).
Clean platinum electrode wires before (and after) each electrophoresis run with a wet tissue paper.
Multiphor and flatbed: Move electrodes so that they will rest on the outer edge of the electrode wicks. Connect the cables of the electrodes to the apparatus and lower the electrode holder plate (fig 6). Close the safety lid.
flatbed professional: Clamp the electrodes on their steel bar so that they will rest on the outer edge of the electrode wicks.
Pipet the samples. Then set the Heavy Weight Glass-plate onto the electrodes, close the lid and start the electrophoresis.

Running conditions (10 °C) max settings of a whole gel

Step	**Volt**	(V read)	**mA**	mA read	**W**	**min**
1	200	(200)	20	(10)	10	10
2	375	(375)	30	(20)	20	50
3	475	(475	30	(20)	25	25

Half of the gel: Keep voltage and halve mA and W
or control with Bromophenol band: When the Bromophenol band has reached the anodal wick, then the run is finished

Hot Coomassie R-350 staining:
This hot Coomassie-staining is staining and fixing simultanuously!
The acetic acid for staining and destaining can be of technical quality.

Stock solutions:
staining solution: 0.03% (w/v) Coomassie R-350 (GE 17-0518-01)
2 tablets (= 0.8 g dye substance) in 2.5 l of 12.5% acetic acid
Use fresh solutions only!
destaining solution: 12.5% acetic acid
impregnating solution: 10% (v/v) glycerol

Staining programme: 30 min *fresh* staining solution at 50°C (fume hood!)
while stirring (fig. 8).
3 x 30 min destaining solution in a tray on a rocking platform, fig 9.
(*Tip: final staining and destaining overnight with:*
0.003% Coomassie R-350, 0.002% Ponceau S, 10 % HAc)
30 min impregnating solution (tray).
Air dry overnight

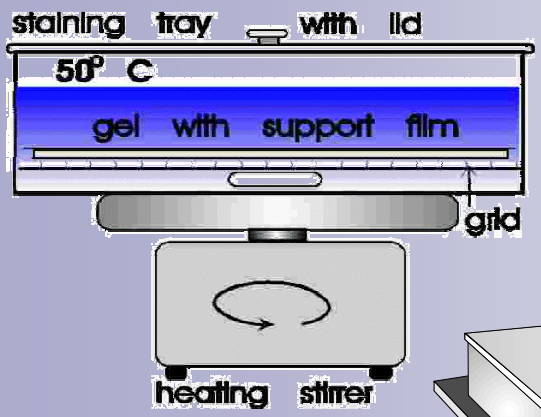


Fig.8: Hot Coomassie-staining

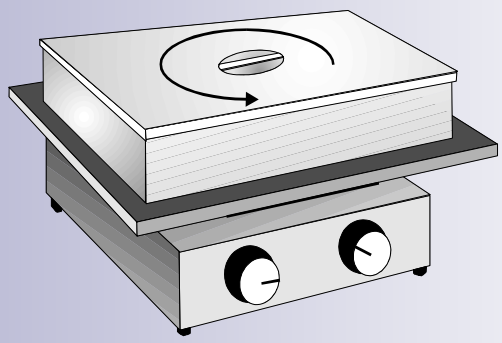


Fig.9: Destaining in a tray

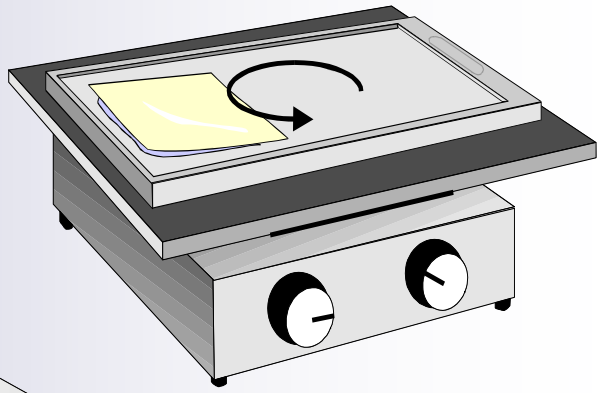


Fig.10: Single step staining

Quick Staining with Azur Gel Super: 1.) Wash the gel for 5 minutes in dist.water to remove tensides and buffers., figure 10. 2.) Stain the gel in the AzurGel Super solution for 30 minutes. 3.) Before air-drying overnight incubate the destained gel in 10% Glycerol for 30 minutes

Silverstaining:
See EDC website for the SDS-silverstaining:
http://www.electrophoresis-development-consulting.de/html/sdssilver.html

References:
[1] Ornstein L. Ann NY Acad Sci. 121 (1964) 321-349
[2] Hermann Schägger: A Practical Guide to Membrane Protein Purification. Academic