Cathodal Native Electrophoresis with the Protein Buffer Kit Cathodic

V8 06/18

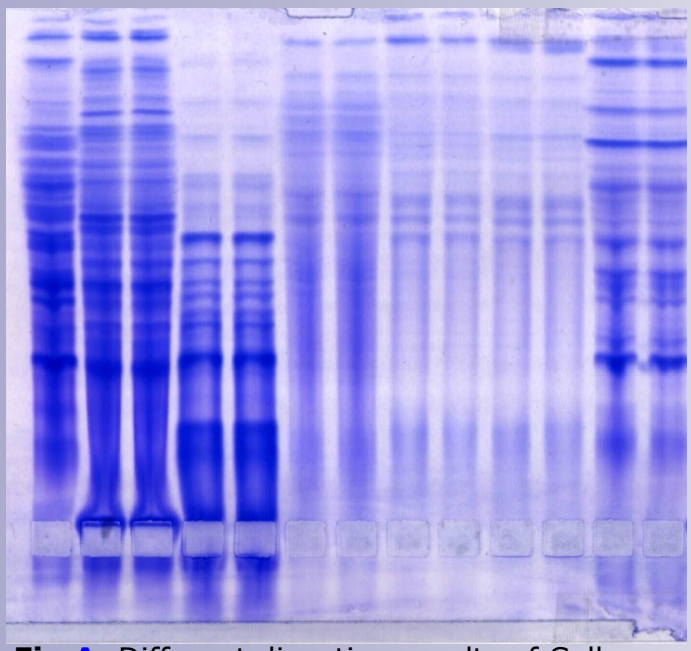


Fig.A: Different digestion results of Collagen -hydrolysate. Cathode is on top! Courtesy of P.Reiter, Gelita, Eberbach (Germany)

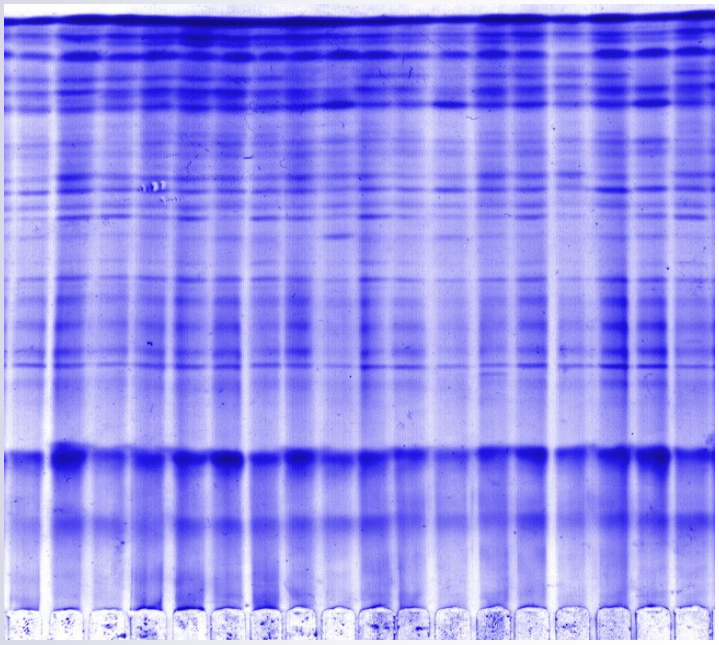


Fig.B: Different maize varieties running cathodal in acidic native electrophoresis incl.3 M urea. Cathode is on top!

General:
This buffer kit is used for acidic electrophoresis with DryGels. A special amphoteric buffer compound establishes a stable pH value of 5.5 in the gel. The additional ions in the buffers are needed for a zone concentration effect for proteins during the start of the electrophoretic separation.
Acidic electrophoresis is applied for separating basic proteins; for instance for separations of lipophilic proteins like alcohol or chloroethanol soluble extracts from barley, wheat, or corn for variety differentiation or identification. The solubility of these proteins can be improved by adding urea and nonionic detergents to the rehydration solution. See figure A and B.

Protein Buffer Kit Cathodic consists of:
Rehydration buffer, **Cathode buffer**, **Anode buffer**
4 Drying Cardboards, Preserving Sheets
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-prof-2836)
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)

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.

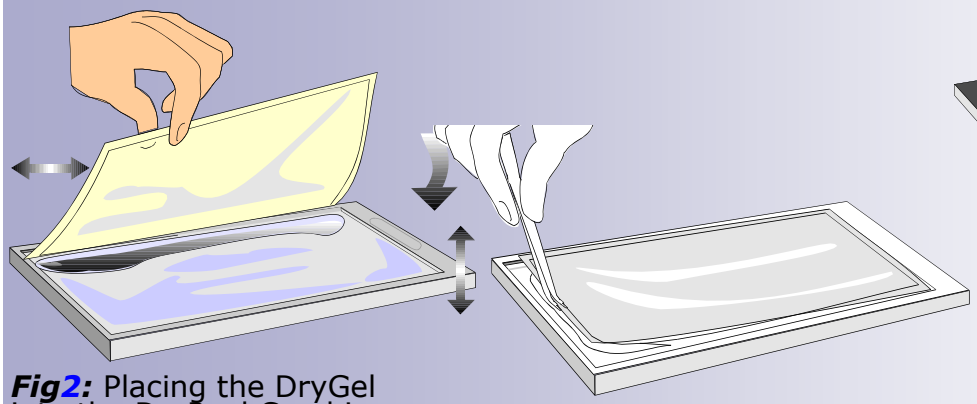
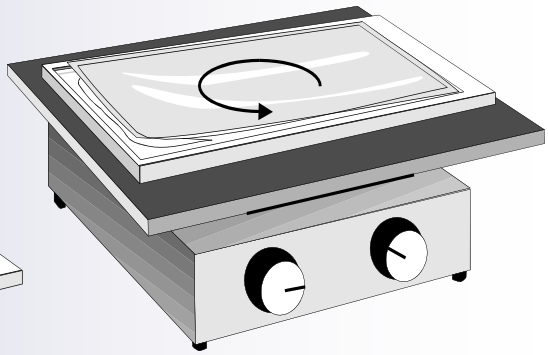


Fig2: Placing the DryGel into the DryPool Combi

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 2.5 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 anode (fig. 6; Multiphor II: cathodal side of the wells matching with line no. 12).
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 cathode buffer contains a blue dye to avoid mistakes (this does not spread evenly in the wick).

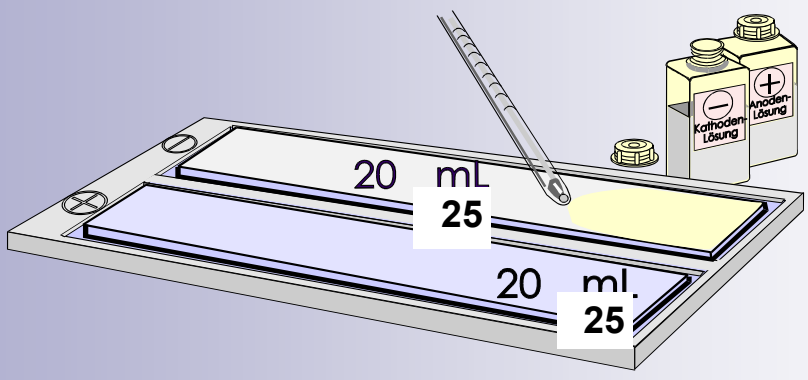


Fig. 4: Soaking the electrode strips with the anode and cathode buffer in the DryPool Combi.

Standards

Sample diluter: Rehydration Buffer 20 ml + dyes: 40 µl Methylenblue (1%), 40 µl Methyl-Green (1%), 100 µl Triton X100 (10%)

1. IEF-standards (3-10 or 7-10) can be used.
2. 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 for Coomassie-staining: 50 µl Protein-Mix + 150 µl, pipet 12.5 µl
3. Cereals: Wheat, Barley - 500 µl / grain

Place the anode strip onto the anodal edge of the gel, matching the grid on the cooling plate (flatbed & Multiphor: between the lines 13 and 14).
Place the cathode strip onto the cathodal edge, matching the grid (flatbed & Multiphor: on line 4). See fig. 5. Always apply anode wick first (more sensitive than the cathode!).
Smooth out air bubbles by sliding bent tip foreceps along the edges of the wicks laying in contact with the gel (first anode, then cathode!).

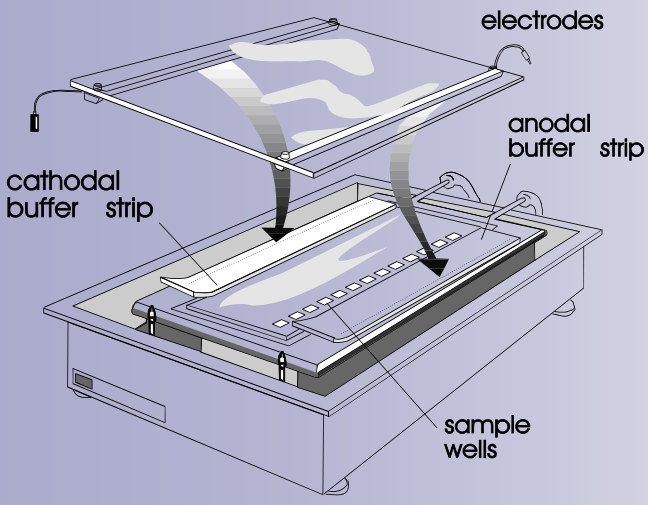


Fig 5: Horizontal electrophoresis apparatus: GE's „Multiphor"

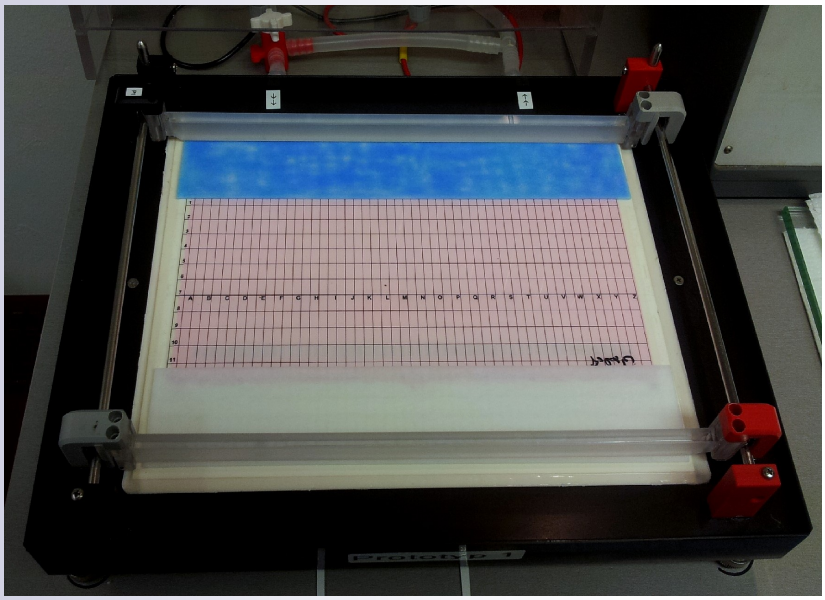


Fig 6: Arrangement of gel, buffer strips and electrodes. Behind the samples is the anode!

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 elctrodes 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 (15 °C*) max settings of a whole gel

Step	**Volt**	(V read)	**mA**	mA read	**W**	**min**
1	300	(120)	7.5	(5)	5	20*
2	500	(340)	15	(10)	10	15
3	800	(800)	20	(20)	20	30
4	900	(850)	25	(25)	20	30

Difficult samples: * sample entrance at 25° using 40V, 80V, 120V and 160V for each 10'. * do not forget to set temperature at your kryostat!
Half of the gel: Keep voltage and halve mA and W
The run is complete, when the red buffer front (pyronin dye marker) has reached the cathodal strip.

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

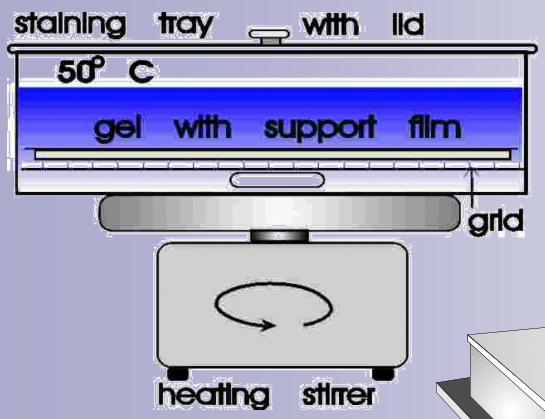


Fig.8: Hot Coomassie-staining

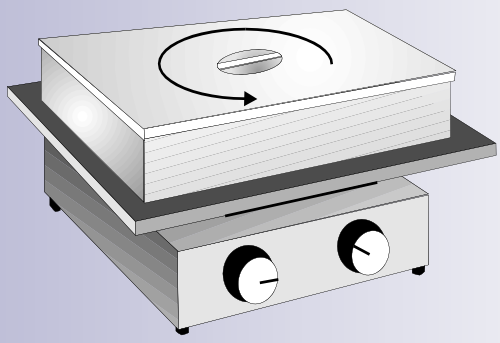


Fig.9: Destaining in a tray

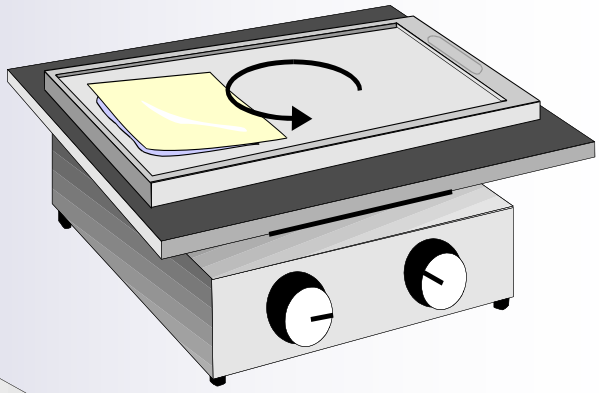


Fig.10: Single step staining

Quick Staining with Azur Gel Super: 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