Cathodal Electrophoresis with Native Buffer Kit pH 5.5

INSTRUCTIONS

CleanGel™ with Native Buffer Kit for horizontal electrophoresis

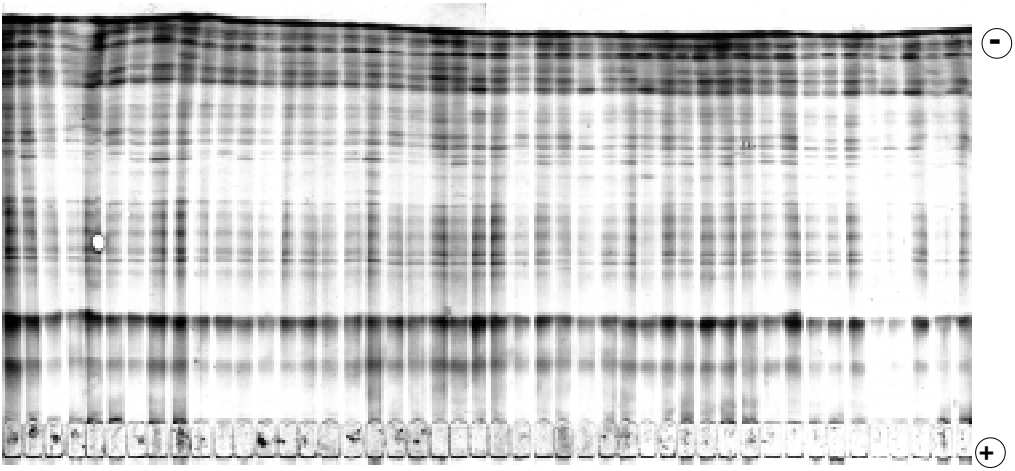

Fig. 1. Cathodal native electrophoresis in acidic buffer system pH 5.5
Samples: Chloroethanol extracts of corn grains in the presence of urea and non-ionic detergent

1. Introduction

Electrophoresis with CleanGel™ system is a very gentle separation method for sensitive proteins and enzymes. CleanGel is available, with different numbers and sizes of sample wells, to suit your particular need. The gels are designed for horizontal electrophoresis, preferably on a Multiphor® II electrophoresis unit.
This buffer kit is suitable for the analysis of proteins with cathodal migration at pH 5.5 e.g. proteins with pI > approx. pH 6.0.

The buffer system has been optimized for washed, rehydratable dry gels. The amphoteric buffer compound HEPES (N-[2-hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]), together with arginine and acetic acid, establishes a stable pH value of 5.5 in the gel. The anodal electrode buffer together with the gel buffer forms a discontinuous buffer system, which contributes to protein zone concentration during the start of the electrophoretic separation.

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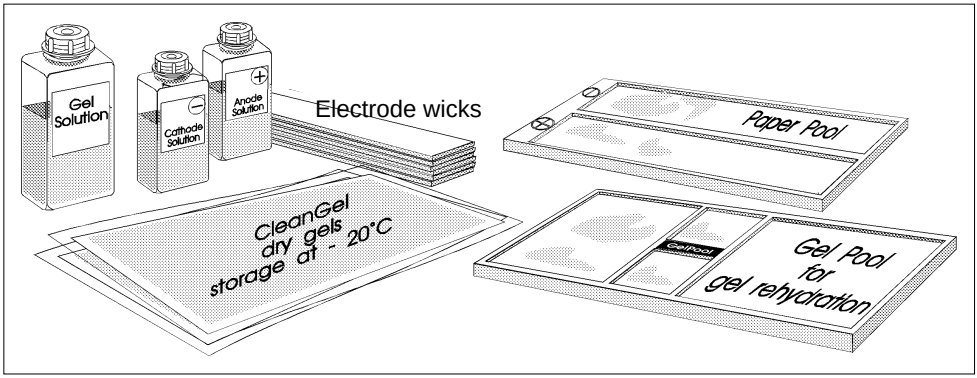

Fig. 2. CleanGel dry gels, Buffer Kit, GelPool and PaperPool.

Package contents and technical data

Package contents

Native Buffer Kit pH 5.5, Code No. 18-1031-631 contains 3 bottles of buffer, electrode wicks and instructions. Buffers and electrode wicks are sufficient for 5 electrophoresis runs.

Technical data

Gel Buffer (Rehydration solution) pH 5.5	0.6 mol/l HEPES 1 mmol/l acetic acid 10 mmol/l arginine 0.01% sodium azide 0.001% Pyronin
Anode Buffer pH 5.8	113 mmol/l ε-aminocaproic acid 5 mmol/acetic acid 0.01% sodium azide
Cathode Buffer pH 3.6	30 mmol/l acetic acid 0.01% sodium azide 0.001% Basic blue
Shelf life:	12 months Please observe the Expiry Date printed on each kit
Storage:	+4 to +8 °C
Electrode wicks:	12 clean paper electrode wicks, 5.5 x 25.3 cm

Designation	No. per pack	Code No.
Gel Buffer	1 bottle (150 ml)	
Anode Buffer	1 bottle (100 ml)	
Cathode Buffer	1 bottle (100 ml)	
Electrode wicks	12	18-1035-33
Instruction	1	71-7138-00

Recommended equipment and accessories

Designation	Code No.
Multiphor II electrophoresis unit	18-1018-06
EPS 3500 XL Power Supply	19-3500-01
MultiTemp III thermostatic circulator, 115 VAC	18-1102-77
MultiTemp III thermostatic circulator, 230 VAC	18-1102-78
Staining kit 1	18-1018-08
CleanGel 25 S	18-1031-54
CleanGel 36 S	18-1031-55
CleanGel 48 S	18-1031-56
GelPool	18-1031-58
PaperPool	18-1031-59

2. Application areas

Cathodic electrophoresis can be used to separate hemolysates, or for the separation of lipohilic proteins such as alcohol or chloroethanol soluble extracts of barley, wheat, and corn for variety differentiation.

3. Sample treatment

Sample concentration

The sensitivity of the detection method used determines the lower limit of the sample amount. Generally, the sample should contain 200 to 500 ng of each component/µl for Coomassie staining, and at least 10 to 25 ng of each component/µl when silver staining is used.
Thumb rule: Total protein concentration, 1-10 µg protein per well for Coomassie staining and 0.05-0.5 µg protein per well for silver staining. Generally, dilute the sample with gel buffer.

Sample preparation

No special sample preparation is necessary, but make sure that all proteins are completely dissolved. Dilute the samples in gel buffer when necessery. The solubility of proteins can be improved by adding urea and non-ionic detergents to the gel buffer.

4. Electrophoresis

Rehydration of CleanGel

Reswell CleanGel with the gel buffer in GelPool according to the instructions with the CleanGel package (Code No. 71-7143-00).

Preparing the experiment

Setting the cooling temperature

Connect Multiphor® II electrophoresis unit to MultiTemp® III thermostatic circulator and set the temperature to +10 °C. Switch on the thermostatic circulator 15 minutes before starting the analysis.

Positioning the gel on the cooling plate

Note: Wear clean gloves to avoid contamination of the gel surface, particularly when using sensitive silver stains.

Pipette about 1 ml of insulating fluid (kerosene or light paraffin oil) onto the cooling plate of Multiphor II. Position the gel in the center of the cooling plate with the sample wells at the a**nodic** side (Fig. 4.). Use the screen print as a guide. No air bubbles should be trapped beneath the gel.

Note: Place the Multiphor II lid in position as soon as possible to prevent drying of the gel.

Applying electrode wicks

Place two of the electrode wicks into the compartements of the PaperPool (if smaller gel portions are used, cut them to the correct size).
Apply 20 ml of the anode buffer to the anodic wick using a pipette (less volume for shorter strips). Perform the same procedure with cathodic buffer and the other wick (Fig. 3).

Note: The cathodic buffer contains a blue dye for easy identification.

Place the anodic wick onto the anodic edge of the gel so that there is a distance of 4 mm between the edge of the wick and the sample wells. Place the cathodic wick onto the cathodic edge of the gel so that the wick overlaps the gel by 5 cm (Fig. 5). **Always apply the anodic wick first.** Smooth out air bubbles by sliding bent tipped forceps along the edges of the wicks lying in contact with the gel (first anode, then cathode!).

Warning: The buffers contain sodium azide (NaN_3) as a preservative.

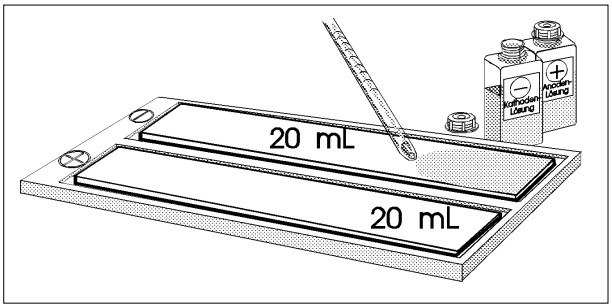

Fig. 3. Soaking the electrode wicks with anodic and cathodic buffers using the PaperPool as a guide.

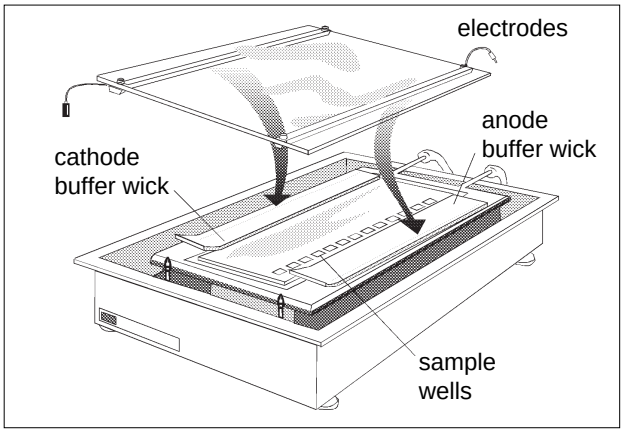

Fig. 4. Arrangement of the gel, buffer wicks and the electrodes on Multiphor II electrophoresis unit.

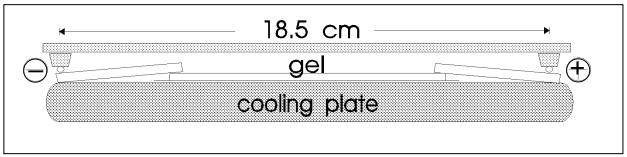

Fig. 5. Sectional drawing, showing the position of the electrode wicks and the electrodes.

Sample application

Apply 5–100 µl sample/well (volume dependent on gel type) by using a micropipette (or use appropriate multipipette, with microtiter plate standard distances).

Running conditions

Clean the platinum electrode wires, before (and after) each electrophoresis run, with a wet tissue paper. Place the electrode holder with the IEF electrodes on the electrophoresis unit. Align the electrodes so that they rest on the outer edge of the electrode wicks (Fig. 5). Connect the cables of the electrodes to the unit. Place the safety lid in position. Connect the power supply. A low starting voltage, for smooth sample entry, generally improves the result. Recommended electrical settings and running conditions are given in Table 1.

Note: If only half of a gel is used, remember to divide the current and power settings by two.

When the buffer front (dye marker Pyronin) reaches the anodic buffer wick, the electrophoresis is complete and should be stopped. Remove the electrode wicks.

5. Detection

All current detection methods used for native electrophoresis can be used with CleanGel. For further information see, for example, Multiphor II electrophoresis system users manual, (Code No. 18-1103-43).

Table 1. Recommended running conditions for one CleanGel

CleanGel pH 5.5	Voltage (V)	Current (mA)	Power (W)	Time (min)
Electrophoresis	500	10	10	10
	1200	28	28	50*

* Approximate time, or until the buffer front (dye marker Pyronin) reaches the anodic wick.

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