# Electrophoresis with SDS buffer kit pH 8.0

*INSTRUCTIONS*

CleanGel[a] with SDS Buffer Kit for horizontal SDS electrophoresis

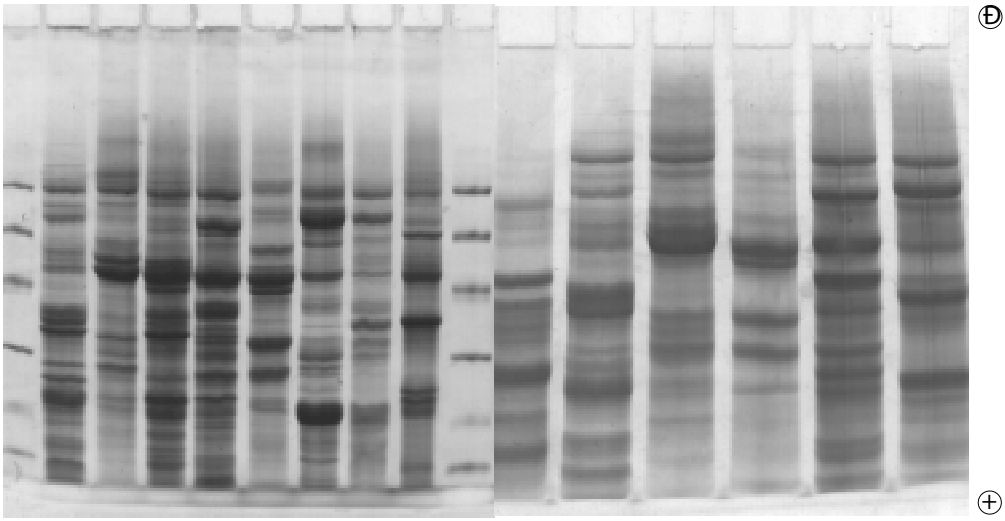

Fig. 1. SDS electrophoresis in rehydrated CleanGel[a] T=5%/10%; Samples: Buffer extract from legume seeds. Staining Comassie blue Left: Sample volume 18 µl each Right: Sample volume100 µl each.

## 1. Introduction

CleanGel[a] is available, with different numbers and sizes of sample wells, to suit your particular need. The gels are designed for horizontal electrophoresis of SDS denatured proteins, preferably on a Multiphor™ II electrophoresis unit. The buffer system, Tris-acetate/tricine, has been optimized for washed, rehydratable dry gels.

The electrode buffers together with the gel buffer form a discontinuous buffer system, which contributes to a protein zone concentration effect during the start of the electrophoretic separation.

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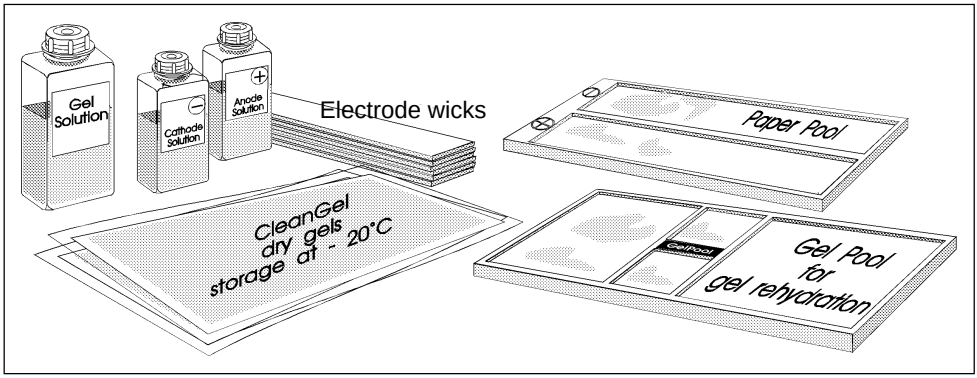


*Fig. 2.* CleanGel dry gels, Buffer Kit, GelPool and PaperPool.

## Package contents and technical data

### Package contents

SDS Buffer Kit pH 8.0, Code No. 18-1031-60, contains 3 bottles of buffer, electrode wicks and instructions. Buffers and electrode wicks are sufficient for 5 electrophoresis runs.

| 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 | 71-7141-00 |

### Technical data

| | |
|---|---|
| Separation range: | 15 - 300 kDa |
| Gel Buffer<br>(Rehydration solution)<br>pH 8.0 | 0.3 mol/l Tris/acetate<br>0.1% SDS<br>0.01% sodium azide<br>0.002% Orange G |
| Anode Buffer<br>pH 8.4 | 0.3 mol/l Tris/acetate<br>0.1% SDS<br>0.01% sodium azide<br>0.005% Bromophenol blue |
| Cathode Buffer,<br>pH 7.15 | 0.8 mol/l Tricine<br>0.08 mol/l Tris<br>0.1% SDS<br>0.01% sodium azide |
| Shelf life: | 12 months<br>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 |

## 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

SDS electrophoresis can be applied to many different studies, for instance: molecular weight determinations of proteins, purity control, separation of denatured proteins, Western blotting and diagnosis of proteinuria.

# 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.

## Sample preparation

When proteins are denatured with excess SDS, the detergent binds to the polypeptides at a constant mass ratio (1.4 g SDS per gram polypeptide) and the polypeptide is organized into a rod-like structure. The bound SDS molecules each contribute a strong negative charge which effectively swamps the intrinsic charge of the polypeptide. The SDS polypeptide complexes have, with a few exceptions, the same charge/mass ratio.
Electrophoretic migration is thus approximately proportional to the molecular weight of the polypeptide chain.

The samples can be treated with SDS, 10 g/l, under non-reducing conditions, reducing conditions or reducing conditions followed by alkylation.

**Non-reducing SDS-treatment**
Dissolve the sample in sample buffer (B) (see Section, Solutions) and heat at 95 °C for at least 3 min. This leaves disulphide bridges between and within the chains intact. SDS-treatment of proteins without reducing agents is common for serum and urinary proteins.

**Reducing SDS-treatment**
Dissolve the sample in sample buffer (C) (see Section, Solutions) and heat at 95 °C for at least 3 min. The disulphide bonds are efficiently reduced by the agent DTT and thus disulphide bridges between and within the chains are broken.

**Reducing SDS-treatment followed by alkylation**
Dissolve the sample in sample buffer (E) (see Section, Solutions) and heat at 95 °C for at least 3 min. After heating, add 10 µl iodoacetamide solution (F) per 100 µl sample.
Alkylation with iodoacetamide prevents possible re-oxidation of free sulfhydryl groups. The bands become very sharp. Proteins with high amounts of cysteine will exhibit an apparent minor increase in molecular weight.

## Solutions

### Sample solutions

**Note:** All chemicals should be of the highest purity. Double distilled water should be used.

| | |
|---|---|
| A. Sample stock buffer: | Dissolve 3.0 g Tris in 40 ml distilled water.<br>Adjust pH to 7.5 with approx. 1.4 ml acetic acid<br>Make up to 50.0 ml with distilled water<br>Storage: 3 months at +4 to +8 °C |
| B. Sample buffer:<br>(with SDS) | 5.0 ml Sample stock buffer (A)<br>0.5 g SDS<br>5 mg Bromophenol blue<br>Make up to 50.0 ml with distilled water, and mix thoroughly<br>Storage: 1 month at +4 to +8 °C |
| C. Sample buffer:<br>(with SDS and DTT) | 5.0 ml Sample stock buffer (A)<br>0.5 g SDS<br>77 mg DTT<br>5 mg Bromophenol blue<br>Make up to 50.0 ml with distilled water, and mix thoroughly<br>Use fresh solution daily |
| D. Sample stock buffer:<br>for alkylation | Dissolve 6.05 g Tris in 40 ml distilled water<br>Adjust pH to 8.0 with 4 mol/l HCl<br>Make up to 50.0 ml with distilled water, and mix thoroughly<br>Storage: 3 months at +4 to +8 °C |
| E. Sample buffer<br>for alkylation | 20 ml Sample stock buffer (D)<br>0.5 g SDS<br>77 mg DTT<br>5 mg Bromophenol blue<br>Make up to 50.0 ml with distilled water and mix thoroughly<br>Use fresh solution daily |
| F. Iodoacetamide solution | Dissolve 100 mg iodoacetamide in<br>500 µl distilled water and mix thoroughly<br>Storage: 2 weeks dark at +4 to+8 °C |

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

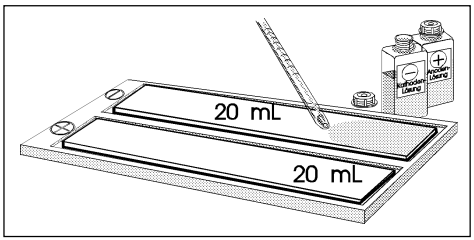


*Fig. 3.* Soaking the electrode wicks with anodic and cathodic buffers using the PaperPool as a guide.

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

Place the cathodic wick onto the cathodic 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 anodic wick onto the anodic edge of the gel so that the wick overlaps the gel by 5 mm (Fig. 5). **Always apply the cathodic wick first.** Smooth out air bubbles by sliding bent tipped forceps along the edges of the wicks lying in contact with the gel (first cathode, then anode!).
**Warning:** The buffers contain sodium azide ($NaN_3$) as a preservative.

## Sample application

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

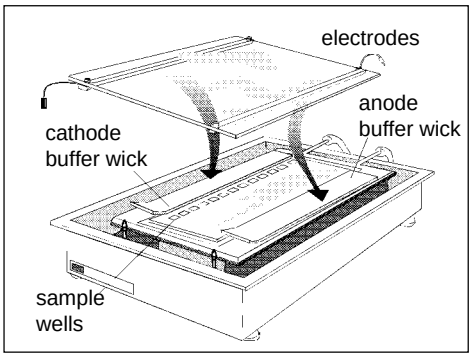


*Fig. 4.* Arrangement of the gel, buffer wicks and the electrodes on Multiphor II electrophoresis unit.

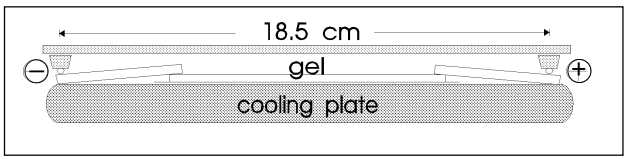


*Fig. 5.* Sectional drawing, showing the position of the electrode wicks and the electrodes.

### 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 lower 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, divide the current and power settings by two.

When the buffer front (dye marker Orange G) 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 SDS electrophoresis can be used with CleanGel SDS. 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 SDS | Voltage (V) | Current (mA) | Power (W) | Time (min) |
|---|---|---|---|---|
| Electrophoresis | 200 | 70 | 40 | 10 |
| | 600 | 100 | 40 | 80* |

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



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