ExcelGel™ SDS Homogeneous 7.5, 12.5 and 15

Polyacrylamide gels and buffer strips for horizontal SDS electrophoresis

Instructions

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1. Introduction

ExcelGel SDS Homogeneous media comprise three homogeneous polyacrylamide gels for horizontal electrophoresis of SDS denatured proteins: ExcelGel SDS Homogeneous 7.5, 12.5 and 15. ExcelGel SDS Homogeneous 15 is also designed for separation of peptides. These gels are also excellent for separation of nucleic acids in the base pair range 50 to 1500. To facilitate handling, the gels are cast on a plastic support film. The gel size is 250 x 110 mm with 25 pre-formed sample wells for sample volumes up to 10 µl/well. The gels are designed for use together with ExcelGel SDS buffer strips. These high quality polyacrylamide strips contain all the buffer needed for SDS electrophoresis and are supplied ready to use. The buffer system in the strips together with the gel buffer form a discontinuous buffer system. The gels are preferably run on the Multiphor™ II electrophoresis unit.

1.1 Package contents and technical data

Package contents

Each gel package contains 6 gels and instructions. The instructions are valid for all three types of gel.

Product	Quantity	Code No
ExcelGel SDS Homogeneous 7.5	6/pkg	80-1260-01
ExcelGel SDS Homogeneous 12.5	6/pkg	80-1261-01
ExcelGel SDS Homogeneous 15	6/pkg	80-1262-01

Technical data

ExcelGel SDS Homogeneous	Stacking gel zone 33 mm	Separating gel zone 77 mm	Separation range kDa
7.5	T5%,C3%	T7.5%,C3%	20-300
12.5	T6%,C3%	T12.5%,C2%	9-210
15	T7.5%,C3%	T15%,C3%	4-170

Gel dimensions:	250 x 110 x 0.5 mm
Buffer in gel:	0.12 mol/l Tris, 0.12 mol/l Acetate and 1 g/l SDS, pH 6.4. ExcelGel SDS homogeneous 15 also contains 30% ethylene glycol
Gel backing:	Polyester film
Shelf life:	12 months
Storage:	2°C to 8°C

1.2 Recommended equipment and accessories

Product	Quantity	Code No
Multiphor II electrophoresis system		18-1018-06
EPS 601 power supply		18-1130-02
MultiTemp™ IV Thermostatic Circulator, 100-120 V		28-9941-72
MultiTemp IV Thermostatic Circulator, 220-240 V		28-9941-71
ExcelGel SDS buffer strips, anode and cathode	6 each/pkg	17-1342-01

1.3 Recommended chemicals

Product	Quantity	Code No
PlusOne Tris	500 g	17-1321-01
PlusOne Sodium dodecyl sulphate, SDS	100 g	17-1313-01
PlusOne Dithiothreitol, DTT	1 g	17-1318-01
PlusOne Formamide	250 ml	17-1320-01
PlusOne Bromophenol Blue	10 g	17-1329-01
PlusOne Glycerol, 87% w/w	1 l	17-1325-01
PlusOne Silver Staining Kit, Protein		17-1150-01
PlusOne DNA Silver Staining Kit		17-6000-30
PlusOne Coomassie Tablets, PhastGel™ Blue R-350	40 tablets	17-0518-01
High Molecular Weight calibration kit, HMW/SDS		17-0615-01
Low Molecular Weight calibration kit, LMW/SDS		17-0446-01
Cellophane preserving sheets	50 sheets	80-1129-38

2. Sample treatment for protein analysis

2.1 Sample concentration

The sensitivity of the detection method used determines the lower limit of the sample amount. Generally, the sample must contain 200 to 500 ng of each component for Coomassie staining, and at least 10 to 25 ng of each component when Silver staining is used. Thumb rule: Total protein concentration, 1 to10 µg protein per well for Coomassie staining and 0.05 to 0.5 µg protein per well when Silver staining is used.

2.2 Sample solutions

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

Sample solutions

Sample stock buffer:	Dissolve 3.0 g Tris in 40 ml distilled water. Adjust pH to 7.5 with approx. 1.4 ml acetic acid. Make up to 50.0 ml with distilled water. Storage: 3 months at 2°C to 8°C.
A. Sample buffer:	5.0 ml Sample stock buffer 0.5 g SDS 5 mg Bromophenol Blue. Make up to 50 ml with distilled water and mix thoroughly. Storage: 1 month at 2°C to 8°C.
B. Sample buffer:	5.0 ml Sample stock buffer 0.5 g SDS 77 mg DTT 5 mg Bromophenol Blue. Make up to 50 ml with distilled water and mix thoroughly. Use fresh solution daily.
C. Sample stock buffer alkylation	Dissolve 6.05 g Tris in 40 ml distilled water. Adjust for pH to 8.0 with 4 mol/l HCl. Make up to 50 ml. Storage: 3 months at 2°C to 8°C.
D. Sample buffer for alkylation	0.5 g SDS 77 mg DTT 5 mg Bromophenol Blue 20 ml sample stock solution (C). Make up to 50 ml with distilled water and mix thoroughly. Use fresh solution daily.
E. Iodoacetamide solution	Dissolve 100 mg iodoacetamide in 500 µl distilled water and mix thoroughly. Storage: 2 weeks in the dark at 2°C to 8°C.

2.3 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 rodlike 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 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 nonreducing conditions, reducing conditions or reducing conditions followed by alkylation.

Non-reducing SDS-treatment

Dissolve the sample in sample buffer (A) and heat at 95°C for at least 3 min. This leaves disulphide bridges between and within the chains intact. This procedure of treating protein samples with SDS 10 g/l without reducing agents is common for serum and urinary proteins.

Reducing SDS-treatment

Dissolve the sample in sample buffer (B) and heat at 95°C for at least 3 min. The disulphide bonds are efficiently reduced by the agent DTT and the disulphide bridges between and within the chains are broken.

Reducing SDS-treatment followed by alkylation

Dissolve the sample in sample buffer (D) and heat for at least 3 min at 95°C. After heating, add 10 µl iodoacetamide solution (E) per 100 µl sample. Alkylation with iodoacetamide prevents possible re-oxidation of free sulfhydryl groups and results in very sharp bands during subsequent electrophoresis. Proteins with high amounts of Cystein may show a minor increase in molecular weight.

3. Sample treatment for DNA analysis

3.1 Sample concentration

The sensitivity of the detection method used determines the minimum amount of sample that can be used.

General rule: The samples should be diluted with sample buffer to about 6 ng of DNA/band per 6 ml application volume when silver staining is used.

3.2 Sample solutions

0.5 mol/l Tris
1.5 g Tris
Dissolve and make up to 25 ml with distilled water.

0.1 mol/l EDTA
1.0 g EDTA disodium salt
Dissolve and make up to 25 ml with distilled water.

1% xylene cyanol
0.1 g xylene cyanol
Dissolve and make up to 10 ml with distilled water.

Sample buffer (total volume: 25 ml)

- 500 µl of 0.5 mol/l Tris solution (final concentration: 10 mmol/l)
- 250 µl of 0.1 mol/l EDTA solution (final concentration: 1 mmol/l)
- 1.25 ml of 1% xylene cyanol solution
- 10 mg of bromophenol blue

- 23 ml of distilled water

Mix thoroughly.
Adjust pH to 7.5 with acetic acid.
Storage: 1 month at 2ºC to 8 ºC.

Denaturing solution (total volume: 25 ml)

- 23.75 ml of 99% formamide
- 1.25 ml of 1% xylene cyanol solution
- 10 mg of bromophenol blue

Storage: 12 months at 2ºC to 8ºC.

Note: If additional heteroduplex banding patterns are to be avoided, the samples can instead be denatured using 50 mmol/l NaOH and 1 mmol/l EDTA disodium salt at 50ºC for 10 minutes.

3.3 Sample preparation

Sample preparation for PCR and DNA analysis

At least 2 µl of sample buffer should be added to each sample (application volume: 6 µl) before electrophoresis.

Mix samples thoroughly and apply to the gel.

Sample preparation for SSCP

Samples are denatured 1:1 in denaturing solution at 95ºC for 5 minutes and thereafter directly placed on ice to prevent reannealing of the single stranded product.

Apply the samples (application volume: 6 µl) to the gel.

4. SDS electrophoresis for protein and DNA analysis

4.1 Preparing the experiment

Setting the cooling temperature

Connect Multiphor II electrophoresis unit to MultiTemp II thermostatic circulator and set the temperature to 15ºC. Switch on the thermostatic circulator 15 minutes before starting the separation.

Positioning the gel on the cooling plate

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

Open the gel package (Fig 1.). Cut around the gel on two sides, about 1 cm from the edge to avoid cutting off the gel or the gel support protruding at the ends. Remove

the gel from the package. If only half the gel is to be used, cut the gel in half with sharp scissors, re-seal the portion to be saved in the package with tape and store it in a refrigerator.

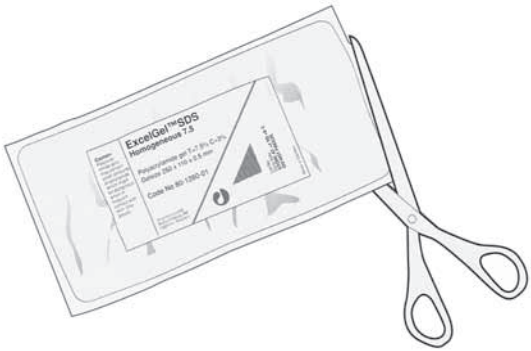

Fig 1.

Take out the gel from the package. The gel is protected with a plastic film and the anodic side is marked with + and an arrow. Carefully remove the thin transparent plastic film from the gel.

Pipette about 1 ml of insulating fluid (kerosene or light paraffin oil) onto the cooling plate of Multiphor II. Position the gel on the cooling plate with the sample wells at th cathodic side. 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 dehydration of the gel.

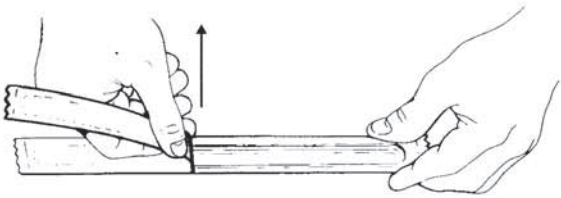

Fig 2.

Applying buffer strips

Open one cathodic and one anodic SDS buffer strip package. Remove the aluminium foil carefully by tearing it step by step, keeping the tearing edge in a perpendicular position (Fig 2.). Remember to use vinyl gloves when removing and applying the buffer strips. If the gloves stick to the buffer strips, moisten the gloves in distilled water. Loosen the strips at one end of the package by putting a spatula between the strip end and the plastic tray (Fig 3.).

Carefully take out the strip from the tray and hold it with both hands about 2 cm away from the ends.

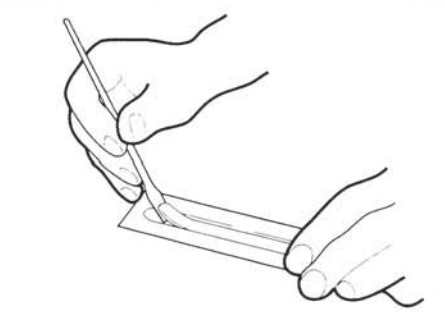

Fig 3.

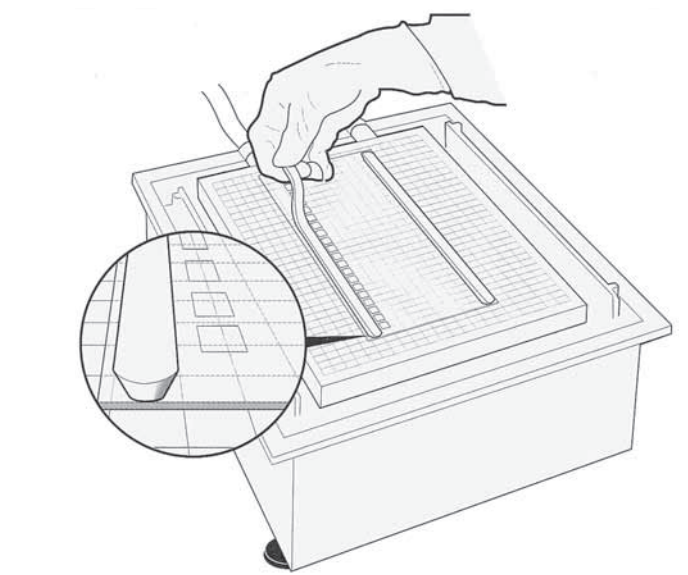

Fig 4.

Apply the cathodic and anodic SDS buffer strips to their respective sides of the gel (Fig 4.).

Note: The narrow side of the strip should be placed on the gel surface. (If the strip breaks, put the pieces together on the gel surface).

4.2 Sample application

Apply 5 to 10 µl sample/well, with a total protein amount of 1 to10 µg/well when Coomassie staining is used and 0.05 to 0.5 µg when Silver staining is used. Apply 6 µl of DNA sample/well when silver staining is used.

4.3 Running conditions

Place the IEF electrode holder on the electrophoresis unit and align the electrodes with the center of the buffer strips (Fig 5.). Place the safety lid in position. Connect the power supply. Follow the recommended electrical settings and running times given in Table 1.

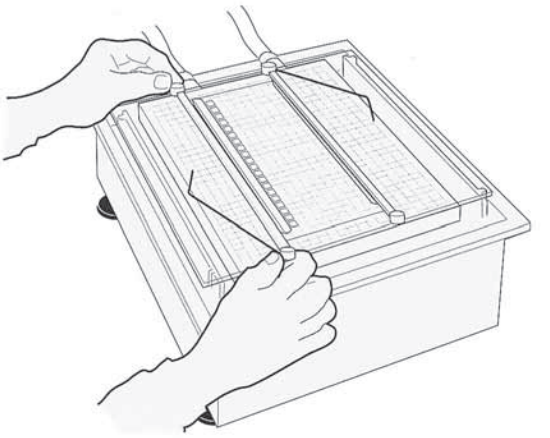

Fig 5.

Table 1. Recommended running conditions for one ExcelGel SDS Homogeneous gel

ExcelGel SDS Homogeneous	**Voltage (V)**	**Current (mA)**	**Power (W)**	**Time (min)**
7.5, 12.5	600	50	30	80*
15	600	30	30	140*

* Approximate time, or until the Bromophenol blue front reaches the anodic buffer strip.

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

When the Bromophenol Blue front reaches the anodic buffer strip the electrophoresis is complete and should be stopped. Remove the buffer strips.

5. Detection

5.1 Silver staining

All current detection methods used for SDS electrophoresis can be used with ExcelGel SDS, homogeneous.

Silver staining is the most sensitive method for permanent staining of proteins and nucleic acids in polyacrylamide gels. Protein and PlusOne DNA Silver Staining Kit contain all components needed for convenient silver staining of proteins and nucleic acids in polyacrylamide electrophoresis gels.

Manual silver staining and Coomassie staining for proteins are described below.

The steps and solutions involved are summarized in Table 2.

Note: 250 ml of each solution is needed per gel for ExcelGel SDS Homogeneous. All steps should be done with gentle shaking of the tray.

Table 2. Silver staining solutions

Fixing solution: 30 min	Ethanol Acetic acid Make up to 1.0 l with distilled water	400 ml 100 ml
Incubation solution: 30 min-overnight	Ethanol Sodium acetate • $3H_2O$ Glutardialdehyde (25% w/v)* Sodium thiosulfate, $Na_2S_2O_3$ • $5H_2O$ Make up to 250 ml with distilled water	75 ml 17.0 g 1.3 ml 0.50 g
Wash: 3 x 5 min	Distilled water	
Silver solution: 40 min	Silver nitrate Formaldehyde* Make up to 250 ml with distilled water	0.25 g 50 µl
Developing solution: 15 min	Sodium carbonate Formaldehyde* Make up to 250 ml with distilled water	6.25 g 25 µl
Stop solution: 2 x 5–10 min	EDTA-Na2 • 2H2O Make up to 250 ml with distilled water	3.65 g
Wash: 3 x 5 min	Distilled water	
Preserving solution: 20 min	Glycerol Make up to 250 ml with distilled water	25 ml

* **Note**: Add these components immediately before use.

Fixation

Immediately after electrophoresis immerse the gel in a staining tray containing fixing solution for 30 minutes. (Do not leave the gel in fixing solution for more than 1 hour, because the gel may come out off the supporting film). This solution precipitates the proteins and allows the SDS to diffuse out of the gel.

Incubation
Place the gel in incubation solution for at least 30 minutes. If necessary, the gel can be left in this solution over night.

Washing
Wash the gel three times in distilled water, each time for 5 minutes.

Silver reaction
Stain the gel for 40 minutes in silver solution.

Developing
Develop the protein bands in developing solution for 15 minutes or until the bands become intensively dark.

Stopping
Stop the reaction by placing the gel in stop solution for 10 to 20 minutes.

Washing
Wash the gel three times in distilled water each time for 5 minutes.

Preserving
Soak the silver stained gel in preserving solutions for 20 minutes. Then place the gel, on glass plate with the gel side up. Cover the gel with cellophane preserving sheet soaked in preserving solution. Allow the gel to dry in room temperature. (Do not put the gel in a heating cabinet, because the silver stain bleaches). To remove excess glycerol from the plastic support after drying the gel, use ethanol or water.

5.2 Coomassie staining

The steps and solutions involved are summarized in Table 3. 250 ml of each solution is used in each step.

Table 3. Coomassie solutions

Fixing solution: 30 min	Ethanol Acetic Acid, HAc Make up to 1.0 l with distilled water	400 ml 100 ml
Staining solution: (Coomassie solution) 10 min	PhastGel Blue R-350 Make up to 400 ml with destaining solution. Heat to 60°C, stirring constantly, and filter before use.	1 tablet

Wash: Rinse once	Distilled water	
Destaining solution: Until the background is clear	Ethanol Acetic Acid, HAc Make up to 1.0 l with distilled water	250 ml 80 ml
Preserving solution: 30 min	Glycerol (87% w/v) 25 ml Make up to 250 ml with destaining solution	

Fixation
Immediately after electrophoresis immerse the gel in a staining tray containing fixing solution for 30 minutes. (Do not leave the gel in fixing solution for more than one hour because the gel may come out off the supporting film). This solution precipitates the proteins and allows the SDS to diffuse out of the gel.

Staining
Stain the gel for 10 minutes in staining solution which has been preheated to 60ºC. Cover the staining dish.

Washing
Rinse the gel once in distilled water.

Destaining
Destain the gel by changing the destaining solution several times until the stained protein bands are clearly visible against the clear background.

Preserving
Soak the destained gel in preserving solution for 30 minutes. Place the gel on a glass plate with the gel side up. Cover the gel with a cellophane preserving sheet soaked in preserving solution. Allow the gel to dry at room temperature or in a ventilated heat cabinet at 50ºC for 1 hour. To remove excess glycerol from the plastic support after drying the gel, use ethanol or water.

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