cytiva

ExcelGel SDS, gradient 8–18

Precast gels and buffer strips for horizontal SDS electrophoresis

Instructions for Use

1 Introduction

ExcelGel™ SDS, gradient 8–18 , is a 0.5 mm-thin precast polyacrylamide gradient gel for horizontal electrophoresis of SDS denatured proteins. This gel is also excellent for separation of nucleic acids in the base pair range 50–1500. To facilitate handling, the gel is cast on a plastic support. The gel size (245 x 110 mm) is sufficient to allow 24–52 samples to be analysed simultaneously depending on the way of sample application. A stacking gel zone on the cathodic side of the gel merges continuously into a separating zone with a linear 8% to 18% polyacrylamide gradient.

The gel is designed for use together with ExcelGel SDS buffer strips (Product code 17134201). 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, forms a discontinuous buffer system. The buffer system and the polyacrylamide gradient gel jointly ensure good separation and sharp bands.

1.1 Package contents and technical data

Package contents

Each gel package contains 6 gels instructions and experimental result forms.

Product code	Designation	No per pack
80125553	ExcelGel SDS, gradient 8–18	6
80131000	Instructions	1
80125509	Experimental result form	6

Technical data

ExcelGel SDS, gradient 8–18	
Stacking gel zone (33 mm):	T=5%, C=3%
Separating gel zone (77mm):	T=8% to 18%, C=3%
Gel dimension:	110 x 245 x 0.5 mm
Separation range:	6.5–300 kDa
	50–1500 bp
Linear separation range:	14–170 kDa
Buffer in gel:	0.12 mol/l Tris, 0.12 mol/l
	Acetate and 1 g/l SDS,
	pH 6.4
Gel backing:	Polyester film
Shelf life:	15 months

Storage:	20°C to 30°C.

1.2 Recommended equipment and accessories

Product code	Designation
18101806	MultiPhor™ II electrophoresis unit with IEF electrode holder
18113005	Electrophoresis Power supply EPS 3500 XL
18110277	MultiTemp™ III 115 V
18110278	MultiTemp III 220 V
17134201	ExcelGel SDS buffer strips
80112946	Sample application pieces
18100274	SDS applicator strip, 26 wells (pkg/5)
18100226	IEF/SDS applicator strip, 52 wells (pkg/5)
80644404	Hoefer Processor Plus Base Unit

1.3 Recommended chemicals

Product code	Designation
80112882	Tris
80112874	Sodium dodecyl sulphate, SDS
80112879	Dithiotreitol, DTT
17132001	Formamide, 250 ml
80112915	Bromophenol Blue
17061501	High Molecular Weight calibration kit, HMW/SDS
17044601	Low Molecular Weight calibration kit, LMW
80112884	Glycerol (87% w/w)
17051801	PhastGel™ Blue R
17115001	PlusOne Silver Staining Kit, Protein
17600030	PlusOne DNA Silver Staining Kit
80112938	Cellophane preserving sheets

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, 1–10 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 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) (see *Section 2.3 Solutions, on page 5*) 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) (see *Section 2.3 Solutions, on page 5*) and heat at 95°C for at least 3 min. The disulphide bonds are efficiently reduced by the reagent 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) (see *Section 2.3 Solutions, on page 5*) 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 the free sulhydryl groups and results in very sharp bands during subsequent electrophoresis. Proteins with high amounts of cystein may show a minor increase in molecular weight.

2.3 Solutions

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

Sample solutions

Sample stock buffer: 0.5 M	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 4°C to 8°C.
A. Sample buffer: non-reducing	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 4°C to 8°C.
B. Sample buffer: reducing	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 for alkylation 1 M	Dissolve 6.05 g Tris in 40 ml distilled water. Adjust pH to 8.0 with 4 mol/l HCl. Make up to 50 ml. Storage: 3 months at 4°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 75 mg iodoacetamide in 500µl distilled water and mix thoroughly. Storage: 2 week in the dark at 4°C to 8°C.

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 µl 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 4°C to 8°C.

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 4°C to 8°C.

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 50°C 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 95°C 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 equipment

Setting the cooling temperature

Connect Multiphor III electrophoresis unit to MultiTemp II thermostatic circulator and set the temperature at 15°C. 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 (see the Figure below). 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. 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 in a refrigerator.

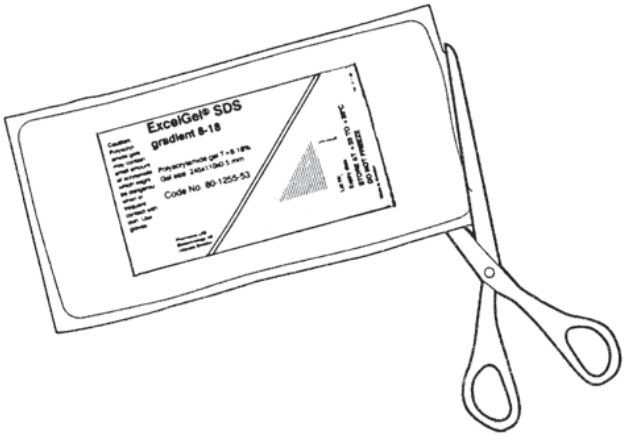

Figure 4.1:

Remove the gel from the package. Note that at the anodic side of the gel, one corner of the supporting film is cut to make it easy to distinguish between the anodic and cathodic sides. 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 so that the polarity of the gel corresponds to that of the plate. 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.*

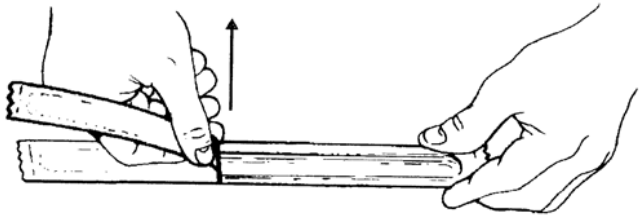

Figure 4.2:

Applying buffer strips

Open a 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 (see the Figure above). 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 (see the Figure below). Carefully take out the strip from the tray and hold it with both hands about 2 cm away from the ends.

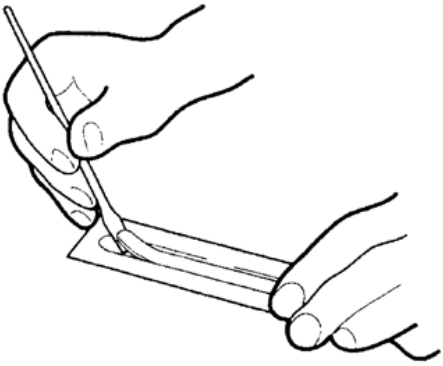

Figure 4.3:

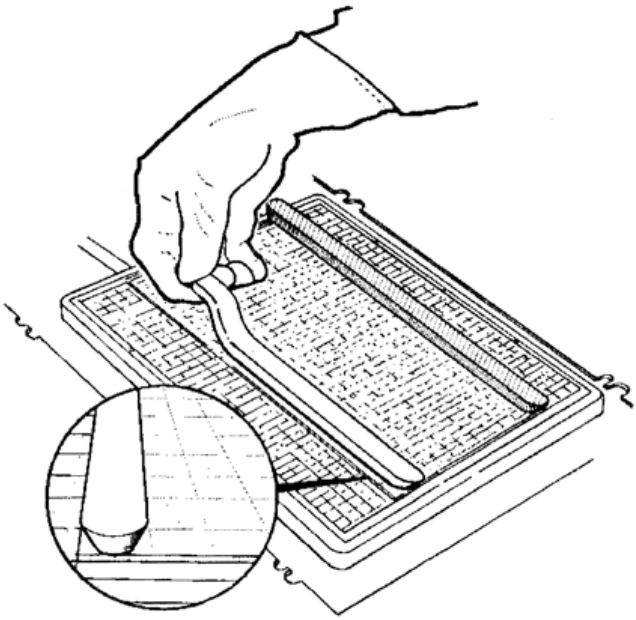

Figure 4.4:

Apply the cathodic and anodic SDS buffer strips to their respective sides of the gel (see the Figure above).

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

4.2 Sample application

The sensitivity of the detection method used determines the lower limit of the sample amount. Preferably, the sample should contain about 200–500 ng of each protein component for Coomassie staining and about 10–25 ng of each component when Silver staining is used. The DNA samples should be diluted with sample buffer to about 6 ng DNA/ band per 6 µl application volume when silver staining is used.

There are three different methods for sample application. The optimal choice depends primarily on the sample and the volume to be applied.

1. *Convenient application of sample volumes, 5–40 µl.*

The sample can be applied directly on the gel by using one of the sample applicator strips. SDS applicator strips (18100274) hold 26 samples with a sample volume of 5–40 µl. IEF/SDS applicator strip (18100226) holds up to 52 samples with a sample volume of 5–20 µl. These sample applicator strips are designed to suit a standard multiple syringe, which makes it easy to apply a large number of samples. (see the Figure below)

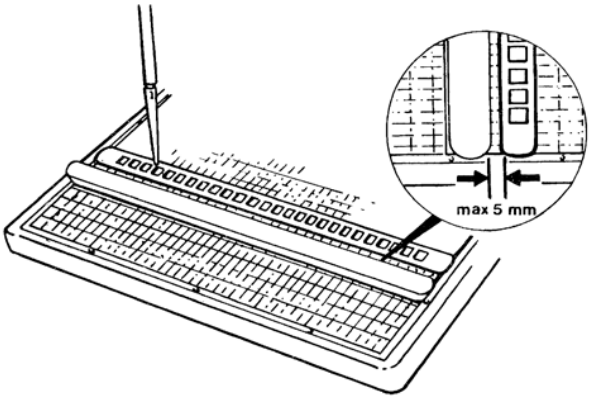

Figure 4.5:

Place the applicator strip about 5 mm from the cathodic buffer strip. Check that the contact between the gel and applicator strip is uniform and apply the samples. *Leave the applicator strip on the gel during electrophoresis.*

2. *For flexible positioning of 5–20 µl samples.*

   Sample application pieces (80112946) hold approximately 20 µl of sample. At least 24 sample application pieces of a size 5 x 10 mm can be applied. For smaller volumes, trim the paper e.g. 2.5 x 5 mm and up to 50 samples can be applied. Place the sample application pieces dry on gel surface about 5 mm from the cathodic buffer strip and apply the samples. *Remove the sample application pieces 15 minutes after the electrophoresis has started.*

3. *Very small sample volumes, up to 3 µl.*

   These can be applied directly on the gel surface as droplets. Apply the samples about 1 cm away from the cathodic buffer strip and at least 1.5 cm away from each short side of the gel. 24 droplets can be applied on one gel.

4.3 Running conditions

Place the IEF electrode holder on the electrophoresis unit and align the electrodes with the centre of the buffer strips. (see the Figure below) Place the safety lid in position. Connect the power supply. Follow the recommended electrical settings and running time given in the table below.

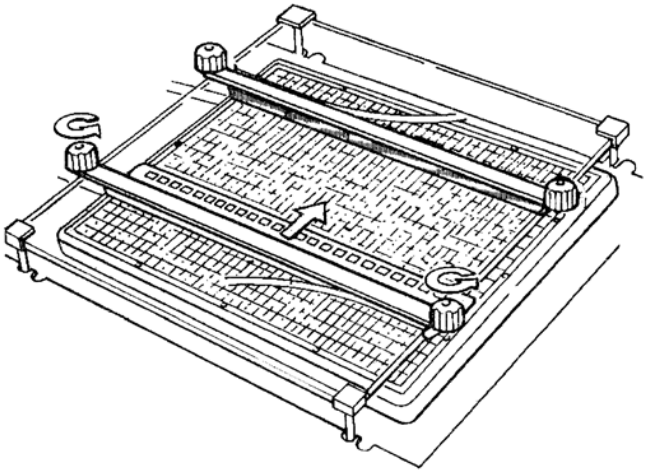

Figure 4.6:

Table 4.1: Suggested running conditions for one ExcelGel SDS, gradient 8–18

	Voltage (V)	**Current (mA)**	**Power (W)**	**Time (min)**
Electrophoresis	600	50	30	80*

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

When the Bromophenol Blue front has reached the anodic buffer strip, the electrophoresis is complete and should be stopped. Remove the buffer strip.

5 Detection

5.1 Silver Staining

All current detection methods used for SDS electrophoresis can be used with ExcelGel SDS, gradient 8–18.

Processor Plus automating staining system is well-suited for silver and Coomassie staining. Silver staining is the most sensitive method for permanent staining of proteins and nucleic acids in polyacrylamide gels. Automation of the silver staining process with Processor Plus and the use of PlusOne Silver Staining Kit, Protein or PlusOne DNA Silver Staining Kit eliminate most of variables associated with silver staining.

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

The Silver staining method is according to Heukeshoven and Dernick (1), with some modifications. The steps and solutions involved are summarized in the table below.

Note: *250 ml of each solution is needed per gel for ExcelGel SDS, gradient 8–18. All steps should be done with gentle shaking of the tray.*

Table 5.1: Silver staining solutions

Fixing solution:	Ethanol	400 ml
30 min	Acetic acid	100 ml
	Make up to 1.0 l with distilled water	
Incubation solution:	Ethanol	75 ml
30 min-overnight	Sodium acetate • $3H_2O$	17.0 g
	Glutardialdehyde (25% w/v)[1]	1.3 ml
	Sodium thiosulfate,	
	$Na_2S_2O_3$ • $5H_2O$	0.50 g
	Make up to 250 ml with distilled water	
Wash:	Distilled water	
3 x 5 min		
Silver solution:	Silver nitrate	0.25 g
40 min	Formaldehyde[1]	50 µl
	Make up to 250 ml with distilled water	
Developing solution:	Sodium carbonate	6.25 g
15 min	Formaldehyde[1]	25 µl
	Make up to 250 ml with distilled water	
Stop solution:	EDTA-Na_2 • $2H_2O$	3.65 g
2 x 5-10 min	Make up to 250 ml with distilled water	

Wash:	Distilled water	
3 x 5 min		
Preserving solution:	Glycerol	25 ml
20 min	Make up to 250 ml with distilled water	

[1] Add these components immediately before use.

Step	Action
1	**Fixation** Immediately after electrophoresis immerse the gel in Multiphor Staining Kit 1 containing fixing solution for 30 minutes. (Do not leave the gel in fixing solution for more than 1 hour, because the gel may come off the supporting film). This solution precipitates the proteins and allows the SDS to diffuse out of the gel.
2	**Incubation** Place the gel in incubation solution for at least 30 minutes. If necessary, the gel can be left in this solution over night.
3	**Washing** Wash the gel three times in distilled water, each time for 5 minutes.
4	**Silver reaction** Stain the gel for 40 minutes in silver solution.
5	**Developing** Develop the protein bands in developing solution for 15 minutes or until the bands become intensively dark. Keep an eye on the gel and stop the reaction when the protein bands are nicely developed.
6	**Stopping** Stop the reaction by placing the gel in stop solution for 10–20 minutes.
7	**Washing** Wash the gel three times in distilled water each time for 5 minutes.
8	**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 the table below.

250 ml of each solution is used in each step.

Table 5.2: Coomassie solutions

Fixing solution:	Ethanol	400 ml
30 min	Acetic Acid, HAc	100 ml
	Make up to 1.0 l with distilled water	
Staining solution:	PhastGel Blue R	1 tablet
(Coomassie solution)	Make up to 400 ml with destaining	
10 min	solution. Heat to 60 °C, stirring constantly, and filter before use.	
Wash:	Distilled water	
Rinse once		
Destaining solution:	Ethanol	250 ml
Until the background	Acetic Acid, HAc	80 ml
is clear	Make up to 1.0 l with distilled water	
Preserving solution:	Glycerol (87% w/v)	25 ml
30 min	Make up to 250 ml with destaining solution	

Step	Action
1	**Fixation** Immediately after electrophoresis immerse the gel in Multiphor Staining Kit 1 containing fixing solution for 30 minutes. (Do not leave the gel in fixing solution for more than one hour because the gel may come off the supporting film). This solution precipitates the proteins and allows the SDS to diffuse out of the gel.
2	**Staining** Stain the gel for 10 minutes in staining solution which has been preheated to 60 °C. Cover the staining dish.
3	**Washing** Rinse the gel once in distilled water.

Step	Action
4	**Destaining** Destain the gel by changing the destaining solution several times until the stained protein bands are clearly visible against the clear background.
5	**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 50 °C for 1 hour. To remove excess glycerol from the plastic support after drying the gel, use ethanol or water.

cytiva.com

For local office contact information, visit cytiva.com/contact

80131000 AI V:8 09/2020