cytiva

# **ExcelGel** 2-D Homogeneous 12.5

## Polyacrylamide gel and buffer strips for flatbed SDS electrophoresis

## Instructions for Use

# 1 Introduction

ExcelGel™ 2-D Homogeneous 12.5 is a 0.5-mm-thick precast polyacrylamide gel for flatbed electrophoresis of SDS denatured proteins, particularly for the second dimension in 2-D electrophoresis separations. To facilitate handling, the gel is cast on a plastic support film. The gel size is 250 × 110 × 0.5 mm. A 6% acrylamide stacking zone on the cathodic side of the gel merges continuously into a homogeneous 12.5% acrylamide separation zone. The gel is designed for use with ExcelGel SDS Buffer Strips. These polyacrylamide strips contain all the buffer needed for SDS electrophoresis and are supplied ready to use. The buffer in the strips together with the buffer in the gel constitute a discontinuous buffer system for improved resolution.

The gel is recommended for use on the MultiPhor™ II Electrophoresis Unit.

These instructions describe the use of ExcelGel 2-D Homogeneous 12.5 for the second dimension separation in 2-D electrophoresis.

Please refer to *Application Note 80644347*, *User Manual 80644366* and *Handbook 80642960* for additional information.

## 1.1 Package contents and technical data

### Package contents

Each gel package contains 6 gels and instructions.

| Designation | No. per pack | Product code |
|---|---|---|
| ExcelGel 2-D Homogeneous 12.5 | 6 | 17600221 |
| Instructions | 1 | 71500903 |

### Technical data

| | |
|---|---|
| Stacking gel zone | 33 mm long, T=6%, C=3% |
| Separating gel zone | 77 mm long, T=12.5%, C=2% |
| Separation range | $M_r$ 10,000-120,000 |
| Gel dimensions: | 250 × 110 × 0.5 mm |
| Gel buffer: | Tris-acetate, pH 6.4 |
| Gel backing: | Polyester film |
| Shelf life: | 15 months |
| Storage: | 4°C to 8°C |

## 1.2 Recommended chemicals and accessories

| Designation | Product code |
|---|---|
| ExcelGel SDS Buffer Strips | 17134201 |
| Multiphor II Electrophoresis Unit | 18101806 |
| Multiphor II Buffer Strip Positioner | 80644290 |
| IEF Sample Application Pieces | 80112946 |
| EPS 1000 Power Supply, 1.0 kV | 18112396 |
| EPS 3500 XL Power Supply, 3.5 kV | 19350001 |
| MultiTemp™ III Refrigerated Bath Circulator, 115 V | 18110277 |
| MultiTemp III Refrigerated Bath Circulator, 230 V | 18110278 |
| Hoefer Automated Gel Stainer with 19 × 29 cm PTFE-coated stainless steel tray. Accepts gels up to 160 × 260 mm. | 80639502 |
| Hoefer Automated Gel Stainer with 29 × 35 cm PTFE-coated stainless steel tray. Accepts gels up to 280 × 260 mm. | 80639616 |
| PlusOne SDS, 100 g | 17131301 |
| PlusOne DTT, 1 g | 17131801 |
| PlusOne Urea, 500 g | 17131901 |
| PlusOne Tris, 500 g | 17132101 |
| PlusOne Glycerol 87%, 1000 ml | 17132501 |
| PlusOne Bromophenol Blue, 10 g | 17132901 |
| PlusOne Silver Staining Kit, Protein | 17115001 |
| LMW Marker Kit | 17044601 |

# 2 Use of ExcelGel 2-D Homogeneous 12.5 in 2-D electrophoresis

Important: To avoid contaminating gels with spurious protein, always wear gloves when handling first dimension strips and ExcelGel SDS gels.

## 2.1 Equilibration of Immobiline DryStrip gels

Run and equilibrate the first dimension Immobiline™ DryStrip gels [immobilized pH gradient (IPG) strips] as described in 2-D Electrophoresis Using Immobilized pH Gradients – Principles and Methods. Equilibrate the IPG strips for 15 minutes in second dimension equilibration solution containing 1% (w/v) DTT followed by 15 minutes more in equilibration solution containing 2.5% iodoacetamide. Three 7-cm long IPG strips can be equilibrated simultaneously in a 15 ml conical centrifuge tube using 10 ml of equilibration solution.

## 2.2 Preparing the Multiphor II and placing the ExcelGel

While the IPG strips are equilibrating for the second dimension, prepare the Multiphor II unit for SDS electrophoresis. Connect the Multiphor II electrophoresis unit to the MultiTemp III thermostatic circulator and set the temperature to 15°C.

Pipette 1.5 ml of kerosene onto the Multiphor II cooling plate. Cut away the edges of the foil packaging for the ExcelGel 2-D Homogeneous 12.5 gel and remove the gel.

A notch identifies the lower-left corner of the gel backing and the anodic (+) end of the gel. Markings on the plastic cover of the gel indicate the direction of electrophoresis. Orient the gel according to these markings and remove the cover of the Multiphor II unit.

Carefully lower the gel onto the cooling plate so that the kerosene spreads completely under the gel backing. The cathodic (–) edge of the gel should line up with the edge of the grid on the cooling plate and the gel should be centred between the right and left edges of the cooling plate (see the Figure below).

***Note:*** *Avoid trapping bubbles between the gel and the cooling plate. Do not allow kerosene to touch the gel surface.*

To improve separation quality, allow the gel to dry slightly by leaving it uncovered for 5–15 minutes before turning on the MultiTemp III Thermostatic Circulator.

*Figure 2.1: ExcelGel on Multiphor II.*

## 2.3 Applying the strip positioner

Place the Multiphor II Buffer Strip Positioner over the gel and cooling plate. The pegs protruding from the bottom of the Strip Positioner should be in contact with the shorter sides of the cooling plate. Use the (–) and (+) symbols to correctly orient the Strip Positioner (see the Figure below).

*Figure 2.2: Placing the Strip Positioner.*

Slide the Strip Positioner so that the cathodic (–) edge of the gel bisects the slot at position 1 (see the Figure below). Lock the Strip Positioner in place by turning the grey locking cam until the Strip Positioner cannot move.

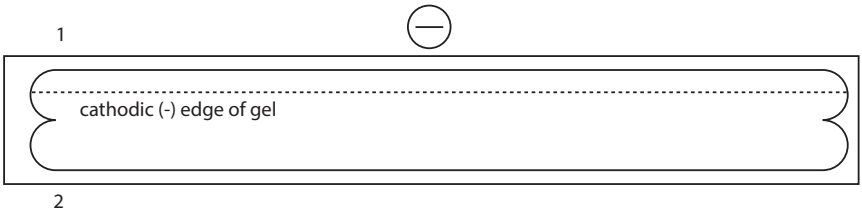


*Figure 2.3: (drawing of slots 1 and 2, and the edge of the gel).*

## 2.4 Applying the buffer strips

***Note:*** *Moisten your gloves with distilled water before removing the buffer strips from their packaging. Always handle the buffer strips by their ends.*

Carefully peel back the foil on the colourless cathodic (–) SDS buffer strip. Place it in the slot at position 1. Make sure that the buffer strip is aligned with the edge of the slot and that the narrow face of the buffer strip is downward (see the Figure below).

Place a few drops of distilled water along the top face of the buffer strip. Stroke the buffer strip with a spatula or fl at forceps to ensure complete contact with the cathodic (–) edge of the gel. The buffer strip should sit snugly within the slot (*Fig. 2.5, on page 8*).

***Note:*** *If a buffer strip breaks, put the pieces together in the Strip Positioner on the gel surface.*

Handle the yellow-coloured (+) anodic buffer strip in the same way and place it in the slot at position 3 (the centre slot) of the Strip Positioner (see the Figure below and *Fig. 2.5, on page 8*).

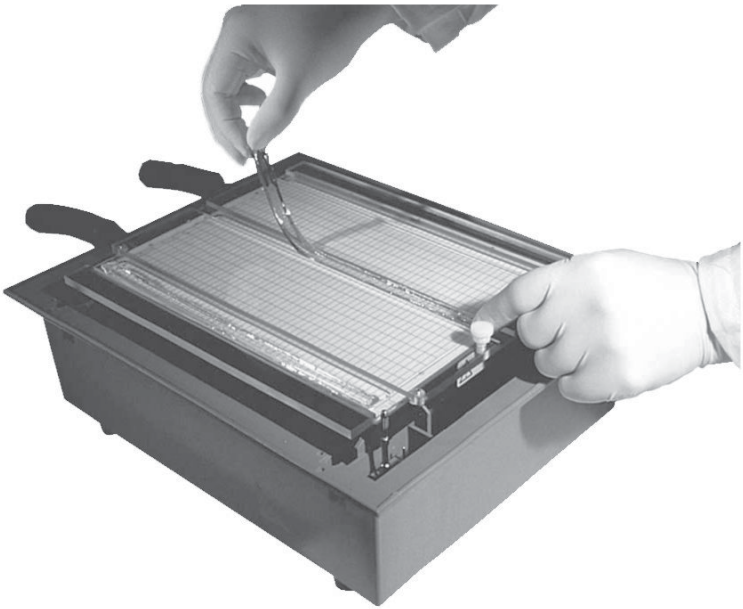

*Figure 2.4: Applying a buffer strip.*

*Figure 2.5: Seating the buffer strip in the slot on the gel.*

## 2.5 Applying the equilibrated IPG strips

Use forceps to remove the IPG strips from the equilibration solution. Remove excess equilibration solution from the surface of the IPG strips by lightly tapping edgewise onto a sheet of moistened filter paper. Alternatively, the IPG strips can be lightly and quickly blotted, gel side down, onto moistened filter paper. The IPG strips can be left resting, gel side up, on the moistened filter paper for up to 10 minutes before proceeding to the next step.

Place the IPG strip(s) gel side down on the ExcelGel through the slot at position 2. The IPG strip(s) should lie in the centre of the slot, parallel to the buffer strip, with approximately 3 mm between the buffer strip and the IPG strip(s). If multiple short IPG strips are being applied to the same second dimension gel, the strips should be in a single, straight line. Leave about at least 1 cm between the ends of each IPG strip (*Fig. 2.6, on page 9*).

Use forceps to place one IEF sample application piece at the ends of each IPG strip underneath the plastic tab formed by the overhanging gel support film at each end of the IPG strip. Be sure that the application pieces touch the ends of the IPG strip (*Fig. 2.7, on page 10*).

*Figure 2.6: Applying the IPG strip.*

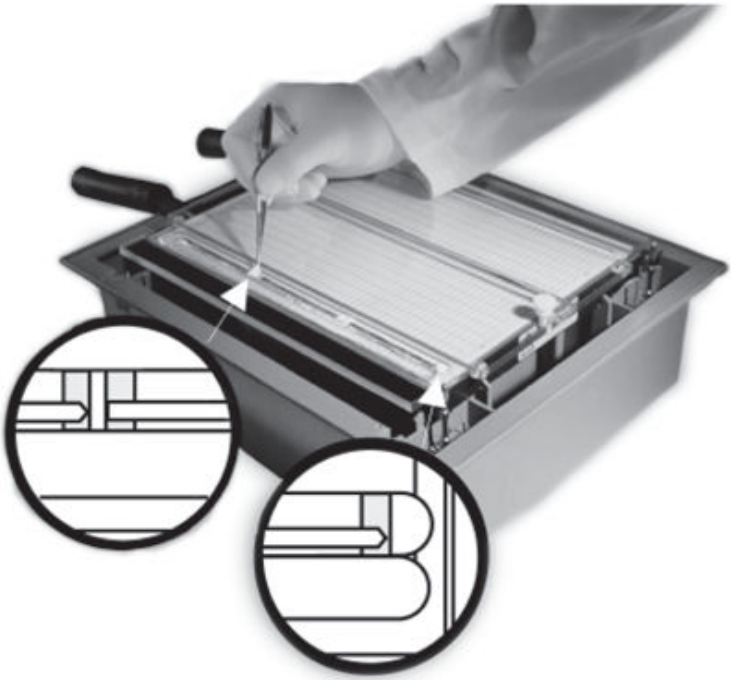

*Figure 2.7: Inserting application pieces at ends of IPG strips.*

***Note:*** *Application pieces absorb any water that flows out of the IPG strips during electrophoresis.*

Make sure that the IPG strip is in full, direct contact with the SDS gel. To remove any air bubbles, stroke the plastic backing of the IPG strip gently with a spatula or a pair of forceps.

To load marker proteins, place an extra application piece (or half piece) on the surface of the gel between two of the IPG strips, or just beyond the end of one of the outer IPG strips. Pipette the marker proteins onto the extra sample application piece. Apply the maker proteins in a volume of 15 to 20 µl. For less volume, decrease the size of the sample application piece proportionally.

## 2.6 Positioning the electrodes

Place the IEF electrode holder on the electrophoresis unit, in the upper (noncontact) position, and align the electrodes with the centre of the buffer strips. Plug in the electrode connectors and carefully lower the electrode holder onto the buffer strips. See the Multiphor II User Manual page 22 and 23.

## 2.7 Running conditions

Place the Safety Lid on the Multiphor II unit. Connect the power supply. Recommended electrical settings and running times are listed in the table below.

***Note:*** *It is important to use a protocol with a low-current sample entry phase. Prior to the second, higher current phase (as indicated in footnote 1 of the table below), remove the IPG strips and application pieces and move the (–) cathodic buffer strip into the slot in the positioner formerly occupied by the IPG strips (position 2). Ensure complete contact of the buffer strip with the gel again by placing a few drops of distilled water along the top surface of the buffer strip and stroking with a spatula or flat forceps.*

Table 2.1: Recommended running conditions for one ExcelGel 2-D Homogeneous 12.5.

| Phase | Voltage (V) | Current (mA) | Power (W) | Duration (h:min) |
|---|---|---|---|---|
| 1 | 600 | 20 | 30 | ~0:35[1] |
| 2 | 600 | 50 | 30 | ~1:15[2] |

[1] When the bromophenol blue dye has moved beyond the slot containing the IPG strips, turn the power off and remove the IPG strips and the application pieces from the gel. Then move the cathodic buffer strip into the slot in the Strip Positioner formerly occupied by the IPG strip(s) (position 2), covering the area of the removed IPG strip(s). Ensure complete contact of the buffer strip with the gel again by placing a few drops of distilled water along the top surface of the buffer strip and stroking with a spatula or flat forceps. Adjust the position of the cathodic electrode, replace the safety lid and turn the power back on.

[2] Stop electrophoresis just as the bromophenol blue front reaches the anodic buffer strip. Remove and discard the buffer strips.

# 3 Detection

Silver staining is recommended for ExcelGel 2-D Homogeneous 12.5. The Hoefer Automated Gel Stainer automates the gel staining process and, combined with the use of PlusOne Silver Staining Kit, Protein, eliminates most of variability associated with silver staining.

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