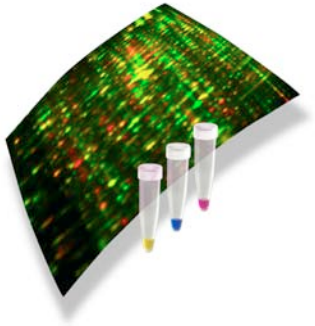
GE Healthcare

Short Instructions 28-9460-86 AA



DIGE Gels and DIGE Buffer Kit

The DIGE gel is a low fluorescent 12.5% polyacrylamide gel for 2-dimensional electrophoresis. The gel is precast in a glass cassette with a buffer providing long shelf-life, up to 12 months.

The DIGE Buffer Kit includes buffer for two DALT*six* electrophoresis runs or one DALT*twelve* electrophoresis run.

For further instructions see:

- *DIGE Gel and DIGE Buffer Kit User Manual* (available as a pdf-file on the web),
- *Ettan*™ *DIGE System User Manual* (18-1173-17 AB), and
- *2-D Electrophoresis Principles and Methods Handbook* (80-6429-60 AC).

Steps

1. Dilute anode buffer in Ettan DALT electrophoresis unit

- For the DALT*six*, insert the gel cassette holder into the tank and add 125 ml (1 bottle) concentrated anode buffer into the buffer tank, rinse the bottle with distilled or deionized water and fill up the tank to the 4.5 l fill line.
- For the DALT*twelve*, set the valve to "circulate" and add 2 × 125 ml (2 bottles) of concentrated anode buffer into the buffer tank, rinse the bottles with distilled or deionized water and fill up the tank to the 7.5 l fill line.

2. Dilute cathode buffer in a separate container

In a separate container, add 2 × 125 ml (2 bottles for DALT*six*) or 4 × 125 ml (4 bottles for DALT*twelve*). Rinse the bottles and fill up with distilled or deionized water to 1.2 l (DALT*six*) or 2.25 l (DALT*twelve*).

3. Set temperature

Set temperature to 15°C (overnight run) or 22°C (day run) and start the circulation pump.

4. Heat sealing solution

Prepare the sealing solution by heating to 95°C in a heating block.

5. Prepare gels

Take out gels from the refrigerator and keep gels at room temperature.

6. Equilibrate Immobiline™ Dry Strip Gels

Equilibrate immobilized pH gradient (IPG) strips in SDS equilibration solution in two steps. Make SDS equilibration buffer: 50 mM Tris-Cl pH 8.8, 6 M Urea, 30% Glycerol (v/v), 2 % SDS (w/v), 0.001% Bromophenol blue (w/v).

a. Prior to use, add 50 mg DTT per 10 ml SDS equilibration solution (0.5% w/v). Place strips in individual tubes or trays and add 10 ml DTT-containing SDS equilibration buffer to each IPG strip. Incubate on a rocker for 15 min.

b. Add 450 mg iodoacetamide per 10 ml SDS equilibration solution (4.5% w/v) instead of DTT. Incubate for an additional 15 min.

Note: *For saturation DIGE dye labeled samples, omit the iodoacetamide step and repeat the first equilibration with DTT-containing SDS equilibration solution for another 15 min.*

7. Load IPG strips on DIGE Gel

Rinse the IPG strip briefly in cathode buffer and carefully place the strip in the glass cassette (see *DIGE Gel and DIGE Buffer Kit User Manual* for further instructions). Gently push the plastic backing to move the strips towards the gel upper surface using a thin ruler or a spatula. Make sure the strip has contact with the gel and avoid trapping air bubbles between strip and gel.

8. Seal the IPG strips in place

Seal the IPG strips in place using an aliquot of hot sealing solution. Carefully pipette across the length of the IPG strip, taking care not to introduce bubbles.

9. Place gels in DALT units and add cathode buffer

- For the DALT*six*, insert gel cassettes into the cassette holder and fill any empty slots with blanks. Wet the upper buffer chamber sealings with some cathode buffer and slide it into place.

  Note: *Do not move the UBC repeatedly up and down.*

  a. Add 1.2 l cathode buffer in the upper buffer chamber and fill up to the fill line.

  b. Using a small funnel placed into the narrow space between the upper and lower buffer chambers, add water or diluted anode buffer to the fill line.

- For the DALT*twelve*, wet gel cassettes and any blanks with some cathode buffer and slide them into the slots. Make sure all slots are occupied and add 2.25 l cathode buffer into the tank and fill up to the fill line.

10. Electrophoresis run conditions

For convenient run times and minimal spot diffusion we recommend the following run conditions.

DALTsix Day Run — Temperature 22°C

Step	mA / gel	Voltage (V)	W/gel	Time (hours)
A	10	80	1	1
B	50	500	17[1]	4-5

1. *Maximum electrical input for the electrophoresis unit is 600 V, 400 mA, and 100 W.*

DALTsix Overnight Run — Temperature 15°C

Step	mA / gel	Voltage (V)	W/gel	Time (hours)
A	12	150	1.5	15-17

DALTtwelve Day Run — Temp. 22°C, constant power

Step	mA / gel	Voltage (V)	W/gel	Time (hours)
A	-	-	1	1
B	-	-	17	4-5

DALTtwelve Overnight Run — Temp. 15°C, constant power

Step	mA / gel	Voltage (V)	W/gel	Time (hours)
A	-	-	1	1
B	-	-	1.5	15-17

11. Stop run

Continue the electrophoresis until the bromophenol blue front reaches the end of the gel. The front can be run off the gel if desired.

12. Scan gels

To minimize spot diffusion the gels should be scanned as soon as possible. Store the gels in a refrigerator in a closed container and keep the gels moist. Allow the gels to reach room temperature before scanning. Keep the gels in the glass cassettes throughout scanning.

Technical specifications

DIGE Gel

Gel composition	T = 12.5%, C = 3% (12.125% acrylamide, 0.375% bisacrylamide)
Separation range	12–120 kDa
Gel dimensions	255 × 196 × 1 mm
Buffer in gel	Special buffer based on piperidinopropionamide (PPA)
Gel cassette	Low fluorescent glass
Shelf life	12 months
Storage	4°C to 8°C

DIGE Buffer Kit

Anode Buffer (2 bottles)	Special buffer based on piperidinopropionamide (PPA)
Cathode Buffer (4 bottles)	0.25 M Tris, 1.92 M glycine, 1% (w/v) SDS
Sealing Solution	Gel Buffer with 0.5% agarose and 0.002% bromophenol blue
Shelf life	Estimated 12 months
Storage	4°C to 8°C

www.gelifesciences.com/proteomics

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

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GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Ltd
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 132, Piscataway, NJ 08855-1327, USA

GE Healthcare Bio-Sciences KK
Sanken Bldg, 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan

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