DIGE Gels and DIGE Buffer Kit

Short Instructions

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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 DALTsix electrophoresis runs or one DALTtwelve electrophoresis run.

For further instructions see:

- *User Manual* 28946089 (available as a pdf-file on the web),
- *User Manual* 18117317, and
- *Principles and Methods Handbook* 80642960.

Steps

Step	Action
1	**Dilute anode buffer in Ettan™ DALT electrophoresis unit**

Option	Action
For the DALTsix	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 DALTtwelve	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.

Step	Action
2	**Dilute cathode buffer in a separate container** In a separate container, add 2 × 125 mL (2 bottles for DALTsix) or 4 × 125 mL (4 bottles for DALTtwelve). Rinse the bottles and fill up with distilled or deionized water to 1.2 L (DALTsix) or 2.25 L (DALTtwelve).
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.

Step	Action
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). • 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. • 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 *User Manual* 28946089 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.

Step	Action

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

Option	Action
For the DALTsix	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.* • *Add 1.2 L cathode buffer in the upper buffer chamber and fill up to the fill line.* • *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 DALTtwelve	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.

Step Action

10 Electrophoresis run conditions

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

Table 1. *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.

Table 2. *DALTsix Overnight Run* — Temperature 15°C

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

Table 3. *DALTtwelve Day Run* — Temp. 22°C, constant power

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

Table 4. *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

Step	Action
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

Table 5. 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

Table 6. 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

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