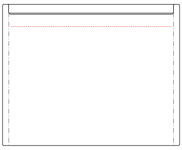
DIGE Gels, DIGE Gels LF24, and DIGE Buffer Kit

Cue Cards

1 Introduction

The DIGE Gels LF24 and DIGE Gels are low fluorescent 12.5% polyacrylamide gels, intended for two-dimensional fluorescence difference gel electrophoresis (2-D DIGE). The precast gels have a shelf life of 12 months. There are two precast gel designs available, the differences are described in the table below.

Precast gel	Compatible with instrument	Gel design
DIGE Gels LF24 (29706670)	• Amersham™ DIGE Unit LF24 • Ettan™ DALTsix • Ettan DALTtwelve	
DIGE Gels (28937451)	• Ettan DALTsix • Ettan DALTtwelve	

The DIGE Buffer Kit includes enough buffer for two Amersham DIGE Unit LF24 electrophoresis runs, two Ettan DALTsix electrophoresis runs, or one Ettan DALTtwelve electrophoresis run.

For further instructions go to *cytiva.com*. The table below describes the user documentation relevant for the different gel designs.

Product	Relevant user documentation
DIGE Gels LF24	• *Amersham DIGE Unit LF24 Operating Instructions, 29701249* • *Ettan DALTsix and Ettan DALTtwelve Systems Operating Instructions, 28964096* • *DIBE coverage analysis Instructions for Use, 29657336* • *2-D Electrophoresis Principles and Methods Handbook, CY14825*
DIGE Gels	• *DIGE Gel and DIGE Buffer Kit User Manual, 28946089* • *Ettan DALTsix and Ettan DALTtwelve Systems Operating Instructions, 28964096* • *DIBE coverage analysis Instructions for Use, 29657336* • *2-D Electrophoresis Principles and Methods Handbook, CY14825*

2 Operation

Step	Action
1	Equilibrate the gels to room temperature.
2	For each gel, heat up one aliquot of sealing solution to 95°C in a heating block.
3	Dilute the anode buffer in the tank:

System	Action
DIGE Unit LF24	Pour 125 mL concentrated anode buffer (1 bottle) into a large beaker. Rinse the bottle with distilled or deionized water, and pour it in the beaker. Fill up the beaker to 4.5 L with distilled or deionized water. Then, pour the buffer into the tank.

Step	Action

System	Action
DALTsix	Insert the gel cassette holder into the tank and pour 125 mL (1 bottle) concentrated anode buffer into the tank. Rinse the bottle with distilled or deionized water. Fill up the tank to the 4.5 L fill line with distilled or deionized water.
DALTtwelve	Set the valve to **circulate** and pour 2 × 125 mL (2 bottles) concentrated anode buffer into the tank. Rinse the bottles with distilled or deionized water. Fill up the tank to the 7.5 L fill line with distilled or deionized water.

4 Dilute the cathode buffer in a separate container:

System	Action
DIGE Unit LF24 and DALTsix	Pour 800 mL distilled or deionized water into a graduated cylinder. Then, pour 250 mL of concentrated cathode buffer (2 bottles) into the graduated cylinder. Rinse the bottles with distilled or deionized water, and pour it into the graduated cylinder. Slowly, fill up the graduated cylinder to 1.2 L with distilled or deionized water.
DALTtwelve	Pour 4 × 125 mL (4 bottles) concentrated cathode buffer into a separate container. Rinse the bottles and fill up with distilled or deionized water to 2.25 L.

5 Set the temperature of the cooling system:

System	Action
DIGE Unit LF24	For the fast day program, use cooled buffer and/or perform the run in the cold room. For the other programs, cooling is optional.
DALTsix and DALTtwelve	Set the temperature to 15°C (overnight run) or 22°C (day run) and start the circulation pump.

6 Equilibrate the IPG strip:

Step	Action

a. Make IPG equilibration buffer in advance:

6 M Urea

50 mM Tris-HCl (pH 8.8)

30% (v/v) Glycerol

2% (w/v) SDS

0.001% (w/v) Bromophenol blue

Store aliquots at -20°C.

b. Prior to use, make two IPG equilibration buffers, one containing DTT and the other containing iodoacetamide, respectively.

Buffer	Action
DTT-containing IPG equilibration buffer	For each IPG strip, dissolve 150 mg DTT in 15 mL of IPG equilibration buffer (1.0% w/v).
Iodoacetamide-containing IPG equilibration buffer	For each IPG strip, dissolve 375 mg iodoacetamide in 15 mL of IPG equilibration buffer (2.5% w/v).

c. Place the IPG strips in individual tubes or trays and add 15 mL DTT-containing IPG equilibration buffer to each IPG strip.

d. Incubate on an orbital shaker or rocking platform for 15 min.

e. Replace the DTT-containing IPG equilibration buffer with 15 mL iodoacetamide-containing IPG equilibration buffer.

f. Incubate on an orbital shaker or rocking platform for 15 min.

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

g. Rinse the IPG strip briefly in cathode buffer.

7 Place the IPG strip on top of the gel:

a. Place the IPG strip on top of the gel in the glass cassette. Gently push the plastic backing to move the IPG strip towards the gel upper surface using a thin ruler or a spatula. Make sure the entire IPG strip has contact with the gel and avoid trapping air bubbles between the IPG strip and the gel.

Step	Action
	b. Seal the IPG strip in place using 1 mL hot sealing solution. Carefully pipette across the whole length of the IPG strip, taking care not to introduce bubbles.
8	Place gels in the tank and pour in the cathode buffer:

System	Action
DIGE Unit LF24	Insert gel cassettes into the gel cassette holder with the notched glass plate facing inward. When running only one gel, fill the other slot with the dummy plate. Place the gel cassette holder in the tank. Make sure that the polarity of the gel cassette holder matches the polarity of the tank. Pour 1.2 L cathode buffer in the inner buffer chamber.
DALTsix	Insert gel cassettes into the gel cassette holder and fill any empty slots with dummy plates. Wet the upper buffer chamber sealings with some cathode buffer and slide it into place. ***Note:*** *Do not move the upper buffer chamber repeatedly up and down.* Pour 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.
DALTtwelve	Wet gel cassettes and any dummy plates with some cathode buffer and slide them into the slots. Make sure all slots are occupied. Add 2.25 L cathode buffer into the tank and fill up to the fill line.

9	Place the safety lid on the tank.
10	Plug in the power leads to the electrophoresis power supply. Make sure that the polarity of the power leads matches the polarity of the electrophoresis power supply.

Step	Action
11	Run the gels with the following run conditions:

Table 2.1: DIGE Unit LF24

Program	Run phase	Voltage (V)[1]	Current (mA)[1]	Power (W/gel)[1]	Time (h)	Cooling
Day run	1	800	400	1	1	N/A
	2	800	400	17	4 to 5	N/A
Fast day run	1	800	400	1	1	Yes[2]
	2	800	400	50	1.5 to 2	Yes[2]
Overnight run	1	800	400	1	8	N/A
	2	80	400	1	9 to 11	N/A

[1] The maximum electrical input for DIGE Unit LF24 is 800 V, 400 mA, and 100 W.
[2] Use cooled buffer and/or perform the run in the cold room.

Table 2.2: *DALTsix*

Program	Run phase	Voltage (V)[1]	Current (mA/gel)[1]	Power (W/gel)[1]	Time (h)	Temperature
Day run	1	80	10	1	1	22°C
	2	500	50	17	4 to 5	22°C
Overnight run	N/A	150	12	1.5	15 to 17	15°C

[1] Maximum electrical input for DALTsix is 600 V, 400 mA, and 100 W.

Table 2.3: *DALTtwelve*

Program	Run phase	Voltage (V)[1]	Current (mA/gel)[1]	Power (W/gel)[1]	Time (h)	Temperature
Day run	1	-	-	1	1	22°C
	2	-	-	17	4 to 5	22°C
Overnight run	1	-	-	1	1	15°C
	2	-	-	1.5	15 to 17	15°C

[1] Maximum electrical input for DALTtwelve is 600 V, 400 mA, and 100 W.

12	Stop the electrophoresis run when the bromophenol blue-front reaches the end of the gel. The front can be run off the gel if needed.

Step	Action
13	Scan the gels. Keep the gels in the glass cassettes during scanning. ***Note:*** *To minimize spot diffusion the gels should be scanned as soon as possible.*
14	Optional: Add a paper tissue soaked in distilled water to prevent the gels from drying out. Store the gels at 4°C to 8°C in a closed container, protected from light.

3 Technical specifications

Table 3.1: DIGE Gels LF24 and DIGE Gels

Parameter	DIGE Gels LF24	DIGE Gels
Gel composition	T = 12.5%, C = 3% (12.125% acrylamide, 0.375% bisacrylamide)	T = 12.5%, C = 3% (12.125% acrylamide, 0.375% bisacrylamide)
Separation range	12–120 KDa	12–120 KDa
Gel dimensions	255 × 186 × 1 mm	255 × 196 × 1 mm
Buffer in gel	Special buffer based on piperidinopropionamide (PPA)	Special buffer based on piperidinopropionamide (PPA)
Gel cassette	Low fluorescent glass	Low fluorescent glass
Shelf life	12 months	12 months
Storage	4°C to 8°C	4°C to 8°C

Table 3.2: DIGE Buffer Kit

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

4 Recycling information

Safety precautions

CAUTION

Always use appropriate personal protective equipment when decommissioning the equipment.

Decontamination

The product must be decontaminated before decommissioning. All local regulations must be followed with regard to scrapping of the product.

Disposal of the product

When taking the product out of service, the different materials must be separated and recycled according to national and local environmental regulations.

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