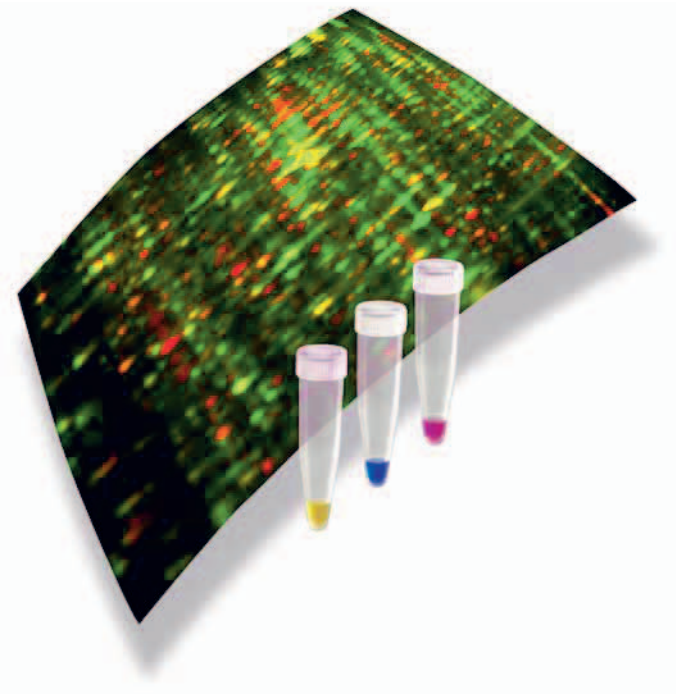
GE Healthcare

DIGE Gel and DIGE Buffer Kit

Precast polyacrylamide gels and buffers for 2-dimensional electrophoresis

User Manual



Important user information

All users must read this entire manual to fully understand the safe use of DIGE Gel and DIGE Buffer Kit.

WARNING!

The WARNING! sign highlights instructions that must be followed to avoid personal injury. Do not proceed until all stated conditions are clearly understood and met.

CAUTION!

The CAUTION! sign highlights instructions that must be followed to avoid damage to the product or other equipment. Do not proceed until all stated conditions are met and clearly understood.

Note

The Note sign is used to indicate information important for trouble-free and optimal use of the product.

CE Certifying

This product meets all requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.

The **CE** symbol and corresponding declaration of conformity, is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked GE Healthcare instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from GE Healthcare except for alterations described in this manual.

Contents

1 Introduction

DIGE Gel is a precast polyacrylamide gel for the second dimension of 2-dimensional (2-D) electrophoresis. The gel is cast in a low fluorescent glass cassette that is compatible with 2-D DIGE analysis. The gel size is 255 × 196 × 1 mm.

The gel is a homogeneous 12.5% polyacrylamide gel cross-linked with bisacrylamide. It is intended to be used in the Ettan™ DALT*twelve* and Ettan DALT*six* electrophoresis units together with the DIGE Buffer Kit. The gel is formulated for long shelf-life and, when used with the DIGE Buffer Kit, generates a discontinuous buffer system offering rapid runs with sharp, reproducible results. The performance and capacity of this gel and buffer system are similar to the widely used Laemmli (Tris-glycine) buffer system.

This User Manual describes how to use DIGE Gel together with the DIGE Buffer Kit for the second dimension of 2-D electrophoresis.

Note: *The gels are not bound to the glass, and are intended to be kept in the glass cassettes through electrophoresis and scanning. For preparative gels and consecutive spot picking we recommend the backing-supported DALT gels* (17-6002-36) *or the Ettan Spot Picker Nonbacked Gel Kit (11-0002-93), see Section 3.13.*

2 Description of the system

DIGE Gel is a precast polyacrylamide gel for the second dimension of large-format 2-D electrophoresis. It is cast in a low fluorescent glass cassette which is compatible with 2-D DIGE analysis. The gel is intended for use in the Ettan DALT*twelve* and Ettan DALT*six* electrophoresis units. The gel is used together with the DIGE Buffer Kit, which includes concentrated buffers for running the gel, and Sealing Solution for attaching the IPG strip to the top of the slab gel.

Ettan DALT*twelve* and DALT*six* units are electrophoresis instruments designed for the second dimension of large-format 2-D electrophoresis using either 24 cm or 18 cm IPG strips. The electrophoresis units can accommodate up to 12 gels or up to 6 gels respectively, either precast or lab-cast.

The buffer system used in the gel gives longer shelf-life than the conventional Laemmli (Tris-glycine) system while retaining the capacity and robustness of that system. Separations performed using the DIGE Gel are similar to those seen with a 12.5% Laemmli gel.

The DIGE Buffer Kit contains all the reagents necessary for a single run of up to 12 DIGE Gels in the Ettan DALT*twelve* electrophoresis unit and two runs of up to 6 gels in the Ettan DALT*six* electrophoresis unit, see Fig 2-1.

Fig 2-1. DIGE Gel and DIGE Buffer Kit.

3 Instructions for use

WARNING! Always wear gloves, protective clothing, and eye protection when handling DIGE gels, DIGE Buffer, IPG strips, gel cassettes or any other equipment these items will come into contact with.

Note: *Always use the highest quality reagents and the purest water available.*

Note: *Do not store opened bottles of DIGE Buffer.*

3.1 Storage of gels

Store the gels horizontally in the original packaging at 4°C to 8°C.

3.2 Preparing samples and first-dimension IEF

For instructions in preparing samples for 2-D electrophoresis and running first-dimension IEF, see 2-D Electrophoresis – Principles and Methods (code no. 80-6429-60 AC) and Ettan DIGE System User Manual (18-1173-17 AB).

3.3 Preparing Ettan DALTtwelve electrophoresis unit

Instructions for using Ettan DALT*twelve* Electrophoresis Unit can be found in the instrument's User Manual.

1 In a separate container dilute the concentrated cathode buffer included in the DIGE Buffer Kit to working strength by adding four bottles of DIGE cathode buffer (total volume 500 ml) and fill up with distilled or deionized water to a total volume of 2.25 l.

2 Ensure that the valve on the separation unit is set to "circulate". Add the entire contents of two bottles of DIGE anode buffer stock solution included in the DIGE Buffer Kit into the tank (see Fig 3-1). Rinse the bottles with distilled or deionized water and pour it into the tank. Fill the tank to the 7.5 l fill line with distilled or deionized water, in this way washing the DIGE anode

buffer from the buffer seal.

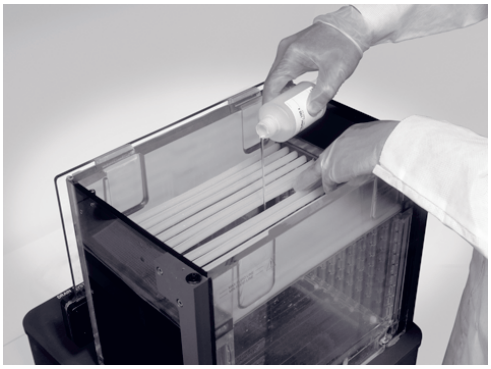


Fig 3-1. Add the anode buffer.

Note: *Avoid pouring the DIGE anode buffer onto the tubing by spreading the tubing elements apart using one hand while pouring the solution with the other hand.*

3 Switch the separation unit on.

4 Turn the pump on to mix, set separation unit to desired temperature. A temperature of 22ºC is recommended for day runs, and 15ºC for overnight runs.

3.4 Preparing Ettan DALTsix electrophoresis unit

Instructions for running the Ettan DALT*six* electrophoresis unit can be found in the instrument's User Manual.

1 When preparing to run a gel in the DALT*six* instrument, insert the anode assembly in the tank and then fill the lower buffer chamber tank. Add one bottle (125 ml) of DIGE Anode Buffer stock solution included in the DIGE Buffer Kit into the tank. Fill the electrophoresis unit to the 4.5 l fill line with distilled or deionized water, and turn the pump on.

Note: *Use only one bottle of the DIGE Anode (lower) Buffer for each DALTsix run.*

2 Turn on the circulation pump.

3 Connect an external MultiTemp™ III thermostatic circulator and set the temperature to 22ºC for a day run and to 15ºC for an overnight run. Equilibrate the buffer to 15ºC before starting an overnight run.

3.5 Equilibrating Immobiline DryStrip gels

The equilibration step saturates the Immobiline™ DryStrip gel (IPG strip) with the SDS buffer system required for the second-dimension separation. To reduce vertical streaking in the second dimension it is necessary to apply two equilibration steps. The first step saturates the IPG strip with the SDS system and the second step blocks the protein thiol groups. The equilibration solution contains buffer, urea, glycerol, reductant, SDS and dye.

Prepare equilibration solution. Prepare SDS equilibration buffer (below). This is a stock solution. Just prior to use, add 50 mg DTT per 10 ml SDS equilibration buffer (0.5% [w/v]).

SDS equilibration buffer

	Final concentration	**Amount**
1.5 M Tris-Cl pH 8.8	50 mM	6.7 ml
Urea (FW 60.06)	6 M	72.07 g
Glycerol (87% v/v)	30% (v/v)	69 ml
SDS (FW 288.38)	2% (w/v)	4.0 g
Bromophenol blue	0.001% (w/v)	2 mg
Distilled or deionized water		to 200 ml

Store in 40 ml aliquots at –20ºC.

Equilibration. Place the IPG strips in individual tubes with the support film toward the tube wall. Add 10 ml DTT-containing solution to each tube. Place the tubes on a rocker and equilibrate for 15 min.

Note: *When using CyDye™ DIGE saturation dyes (Labeling Kit for scarce samples), repeat the first equilibration with DTT-containing SDS equilibration solution for another 15 min.*

Second equilibration. A second equilibration is performed with an iodoacetamide solution (instead of DTT). Prepare a solution of 450 mg iodoacetamide per 10 ml of SDS equilibration buffer (4.5% [w/v]). Decant the first equilibration solution and add the same volume of iodoacetamide containing equilibration solution to each tube. Place the tubes on a rocker and equilibrate for an additional 15 min.

Note: *The subsequent steps of electrophoresis unit preparation, insertion of gels and melting of the Sealing Solution can be performed while the IPG strips are equilibrating.*

3.6 Applying Immobiline DryStrip gels

Take out the gels from the refrigerator and keep gels at room temperature. Open the gel package and remove the gel. The DIGE gels come in glass cassettes and are ready to be used. Allow the gels to reach room temperature before use.

For more information about the application of strips, see *Ettan DIGE System User Manual* (18-1173-17 Edition AB).

3.7 Inserting gel cassettes into Ettan DALTtwelve

When the electrophoresis buffer has reached the desired temperature, insert the loaded gel cassettes with the IPG strips in place.

Note: *Gel Cassettes and Blank Cassette Inserts slide much more easily into the unit if they are wet. Wetting the cassette with some cathode buffer using a soaked kleenex or alternatively distilled or deionized water from a squirt bottle can be used to wet the cassettes and Blank Cassette Inserts as they are being loaded into the unit.*

1 Fit Blank Cassette Inserts into any unoccupied slots.

2 Load the unit from back to front.

3 When all 12 slots are occupied, the buffer level should be slightly below the level of the gaskets. If the buffer level is too low, add distilled or deionized water to the lower buffer chamber. If excess anode buffer is in the upper reservoir, remove it with a pipette. Pour the diluted cathode buffer into the tank to the fill line (some of this buffer may drip through the gasket and mix with the anode buffer during the run, but this will not affect performance or results).

3.8 Inserting gel cassettes into Ettan DALTsix

When the electrophoresis buffer has reached the desired temperature, insert the loaded gel cassettes with the IPG strips in place.

1 Wet the UBC sealings with cathode buffer or 0.1% SDS (immerse the sealings in solution or spray the sealings of the UBC using a plant sprayer) and carefully slide the UBC over the gel cassettes.

Note: Do not move the UBC repeatedly up and down as this will reduce the sealing effect.

2 In a separate container, add 2 × 125 ml (2 bottles) of cathode buffer. Rinse the bottles and fill up with distilled or deionized water to 1.2 l.

3 Fill the UBC with 1.2 liters of diluted cathode buffer and use a funnel to adjust the buffer level in the lower buffer chamber to the same height as in the UBC, by adding water or diluted anode buffer.

3.9 Recommended running conditions – Ettan DALTtwelve

Program the Ettan DALT*twelve* electrophoresis unit to deliver the following protocol.

Constant power
Temperature: 22°C

Phase	Power W/gel	Duration
1	1 W	1 hour
2	17 W (max 180 W)[1]	Until the bromophenol dye front reaches the bottom of the gel (approximately 4-5 hours for a full set of 12 gels).

[1] For overnight runs, the power is set to 1 W/gel and the recommended temperature is 15ºC. Alternatively, set the temperature to 22ºC. Run for 1 hour and then increase to 1.5 W/gel.

3.10 Recommended running conditions – Ettan DALTsix

The maximum rated electrical input for the electrophoresis unit is 400 mA, 100 W, and 600 V. For overnight runs we recommend to set the temperature to 15ºC. Alternatively, set the temperature to 22ºC.

For further run conditions, see the *DIGE Gels and DIGE Buffer Kit Short Instructions* (28-9460-86) in the DIGE Buffer Kit.

Run conditions (Day run):

Set the MultiTemp temperature to 22ºC.

Step	mA/gel	Voltage (V)	W/gel	Time (hours:mins)
1	10	80	1	1:0
2	50	500	17[2]	4:00-5:00[3]

[2] The maximum rated input power for the electrophoresis unit is 100 W.

[3] Continue the electrophoresis until the bromophenol blue reaches the end of the gel.

Run Conditions (Overnight):

Set the MultiTemp temperature to 15ºC.

Step	mA/gel	Voltage (V)	W/gel	Time (hours:mins)
1	12	150	1.5	15:00-17:00[4]

[4] Continue the electrophoresis until the bromophenol blue reaches the end of the gel.

3.11 Unloading gels from electrophoresis units

For information regarding unloading gels from the electrophoresis units, please refer to the respective instrument User Manuals. The gels should not be removed from the cassettes.

3.12 Detection

Scan the gels as soon as possible after the second dimension SDS-PAGE is finished in order to minimize protein diffusion. Typhoon™ Variable Mode Imager or Ettan DIGE Imager are recommended for scanning DIGE second dimension SDS-PAGE gels. 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.

3.13 Further analysis of protein spots

For preparative gels and consecutive spot picking, the backing-supported DALT gels (17-6002-36) and the DALT Buffer Kit (17-6002-50) can be used. CyDye DIGE fluor minimal dye Cy™5 can be included for matching and detection purposes. Alternatively, the non-supported DIGE gels can be used with the Ettan Spot Picker Nonbacked Gel Kit (11-0002-93).

DIGE gels can be removed from the glass cassettes for staining or Western Blotting. To open the cassette, use a spatula to remove the glue at one side of the cassette. Open carefully to avoid damaging the gel. Adding small amounts (ml) of water or buffer on top of the gel will aid the opening process. With the gel positioned on one glass plate the DIGE gel can be easily moved to a tray for post-staining, e.g. using Deep Purple™. Please note that the gel is not attached to a glass plate.

4 Troubleshooting

This section concerns troubleshooting problems that have their origin in the second-dimension separations using DIGE Gel. For a more comprehensive guide to troubleshooting problems with 2-D electrophoresis, see *2-D Electrophoresis Using Immobilized pH Gradients – Principles and Methods (80-6429-60)*.

Symptom	Possible cause	Remedy
No current at start of run	Insufficient volume of buffer in upper reservoir.	Ensure that the upper reservoir contains enough buffer to contact the upper electrode.
Buffer not circulating (Ettan DALT*six* only)	Pump is not primed.	Turn pump off and on to purge air bubbles.
	Pump is off.	Turn pump on.
	Pump is broken.	Call for service.
Second-dimension separation proceeds slowly with high current (Ettan DALT-*twelve* only)	All the slots in the sealing assembly are not occupied by either gel cassettes or Blank Cassette Inserts.	Ensure that all 12 slots in the sealing assembly are occupied.
	Anodic buffer has mixed with cathodic buffer from overfilling of either the cathodic reservoir or the anodic reservoir.	Do not pour more than the suggested volume (7.5 l) into the lower reservoir. Ensure that the level of the anode buffer does not come above the sealing assembly when the electrophoresis unit is fully loaded. If excess anode buffer is in the upper reservoir, it should be removed with a pipette. Ensure that the level of cathode buffer does not come above the air vents in the corners of the upper reservoir. Lack of mixing between upper and lower reservoirs can be verified by adding bromophenol blue dye to the lower reservoir prior to loading the unit with gels. Several drops of 1% (w/v) bromophenol blue will impart sufficient color to the anode buffer.

Symptom	Possible cause	Remedy
Second-dimension separation proceeds slowly with high current (Ettan DALT*six* only)	One of the slots in the upper buffer chamber is open.	All six slots in the upper buffer chamber must be occupied by either a gel cassette or a blank cassette.
	Upper buffer chamber is damaged.	Change upper buffer chamber.
	Anodic buffer has mixed with cathodic buffer from overfilling of either the cathodic reservoir or the anodic reservoir.	Ensure that the level of the anode (lower) buffer does not come above the level of the buffer in the upper buffer chamber when the electrophoresis unit is fully loaded.
Dye front is irregular	The top surface of the gel has been damaged during application of the IPG strip.	Take care during application of the IPG strip that the gel is not damaged.
	Interfering substances in the first dimension.	Contaminants in the sample can cause distortions or swollen regions in the IPG strip following IEF. These distortions can result in disturbances in the second dimension. Modify sample preparation to limit these contaminants. Refer to 2-D Electrophoresis Using Immobilized pH Gradients – Principles and Methods (code no. 80-6429-60) for suggestions.
Distortion in the 2-D pattern	Interfering substances in the first dimension.	Contaminants in the sample can cause distortions or swollen regions in the IPG strip following IEF. These distortions can result in turn in disturbances in the second dimension. Modify sample preparation to limit these contaminants. Refer to 2-D Electrophoresis Using Immobilized pH Gradients –Principles and Methods (code no. 80-6429-60) for suggestions.

Symptom	Possible cause	Remedy
Vertical gap in the 2-D pattern	Bubble between IPG strip and top surface of second dimension gel.	Ensure that no bubbles are trapped between the IPG strip and the top surface of second-dimension gel.
Vertical streaking	Incorrectly prepared equilibration solution.	Prepare equilibration solution according to instructions.
	Poor transfer of protein from IPG strip to second gel.	Employ a low power or current sample entry phase in the second-dimension electrophoresis run. Prolong entry phase if necessary.
	Insufficient equilibration.	Prolong equilibration time.
	IPG strip was not equilibrated with iodoacetamide in a second equilibration step.	Equilibrate IPG strip in two steps. 1st step with DTT (0.5%) and 2nd step with iodoacetamide (4.5%) according to instructions on page 9.
Spots are vertically doubled, or "twinned"	IPG strip is not placed properly.	Ensure that the plastic backing of the IPG strip is against the glass plate of the second dimension cassette.
Poor representation of higher molecular weight proteins	Incorrectly prepared equilibration solution.	Prepare equilibration solution according to instructions.
	Poor transfer of protein from IPG strip to second-dimension gel.	Employ a low power or current sample entry phase in the second-dimension electrophoresis run. Prolong entry phase if necessary.
Bubbles lagging after the front		These bubbles do not affect the result.

5 Technical information

5.1 Package contents

DIGE Gel

Each gel package contains three gels.

Designation	No. per pack	Code No.
DIGE Gel	3	28-9374-51

DIGE Buffer Kit

Each kit contains 6 bottles of buffer, 12 tubes of sealing solution, and the Short Instruction. The solutions are sufficient for a single run of up to 12 gels or two DALT*six* runs.

Designation	No. per pack	Code No.
DIGE Buffer Kit	Sufficient to run 12 gels	28-9374-52
Contains:		
Anode Buffer	2 bottles (2 × 125 ml)	
Cathode Buffer	4 bottles (4 × 125 ml)	
Sealing Solution	12 tubes (12 × 1 ml)	

5.2 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

5.3 Recommended equipment, accessories and reagents

Designation	Code No.
Ettan DALT*twelve* Separation Unit and Power Supply/Control Unit, 230 VAC	80-6466-27
Ettan DALT*twelve* Separation Unit and Power Supply/Control Unit, 115 VAC	80-6466-46
Ettan DALT*six* Electrophoresis Unit including buffer circulation pump and Peltier cooling, 230 VAC	80-6485-27
Ettan DALT*six* Electrophoresis Unit including buffer circulation pump and Peltier cooling, 115 VAC	80-6485-08
MultiTemp III Thermostatic Circulator, 115 V	18-1102-77
MultiTemp III Thermostatic Circulator, 230 V	18-1102-78
EPS 601 Power Supply	18-1130-02
DALT Blank Cassette Insert	80-6467-03
Cassette Rack	80-6467-98
PlusOne Urea	17-1319-01
PlusOne Tris	17-1321-01
PlusOne Glycerol	17-1325-01
PlusOne Dithiothreitol	17-1318-01
PlusOne Sodium Dodecylsulfate	17-1313-01
PlusOne Bromophenol Blue	17-1329-01
Equilibration Tube Set (12/pk)	80-6467-79

Immobiline DryStrip Dry polyacrylamide gels (0.5 mm, T = 4%, C = 3%, after rehydration) cast on plastic backing. 12/pk

pH interval	Code No. / 18 cm strip	Code No. / 24 cm strip
3.5–4.5		17-6002-38
3–7 NL		17-6002-43
4–7	17-1233-01	17-6002-46
6–9	17-6001-88	17-6002-47
6–11	17-6001-97	
3–10	17-1234-01	17-6002-44
3–10 NL	17-1235-01	17-6002-45
3–5.6 NL	17-6003-56	17-6003-57
5.3–6.5	17-6003-61	17-6003-62
6.2–7.5	17-6003-66	17-6003-67
7–11 NL	17-6003-71	17-6003-72
3-11 NL	17-6003-76	17-6003-77

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