DIGE Gel and DIGE Buffer Kit

2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) is a key technology for analyzing differences in protein abundance in biological samples. DIGE Gel and DIGE Buffer Kit are tools to increase the quality, reproducibility, and convenience of 2-D DIGE using Ettan™ DIGE System. DIGE Gel is a precast polyacrylamide gel in a low fluorescent glass cassette for second dimension 2-D electrophoresis. The DIGE Buffer Kit is a specially designed two-buffer system for running up to twelve DIGE Gels simultaneously (Fig 1).

DIGE Gel and DIGE Buffer Kit offer:

- *High quality protein spot maps:* DIGE Gel provides high sensitivity and high resolution protein spot maps of equal quality compared to homecast polyacrylamide gels.
- *Reproducibility:* DIGE Gel run with DIGE Buffer Kit result in spot maps with higher reproducibility compared to homecast polyacrylamide gels, reducing technical variation between gels.
- *Convenience:* DIGE Gel and DIGE Buffer Kit are ready-to-use products with a long shelf-life that saves you time by eliminating the need to make buffers, cast gels and wash glass plates, leaving more time for more important work.

Description

DIGE Gel is a 12.5% precast, low fluorescent polyacrylamide gel cast in a low fluorescent glass cassette specially developed for 2-D DIGE analysis. DIGE Gel should be used with the DIGE Buffer Kit, which consists of concentrated running buffers and Sealing Solution for attaching Immobiline™ DryStrip Gels (IPG strips) to the top of the polyacrylamide gel. The capacity of the buffer system is similar to the commonly used Laemmli (Tris-Glycine) buffer system, and the separation performance of DIGE Gel is comparable to 12.5% Laemmli gels (1). Therefore, DIGE Gel and DIGE Buffer Kit offer the high quality and performance of the Laemmli system with the added advantages of higher reproducibility, convenience, and time savings. The main characteristics of DIGE Gel and DIGE Buffer Kit are listed in Table 1.

Fig 1. DIGE Gel and DIGE Buffer Kit for differential protein analysis using the Ettan DIGE System.

Table 1. Characteristics of DIGE Gel and DIGE Buffer Kit

DIGE Gel	
Gel composition	T = 12.5%, C = 3% (12.125% acrylamide, 0.375% bisacrylamide)
Separation range	12 000 to 120 000 M_r, relative molecular mass
Gel dimensions	255 × 196 × 1 mm
Buffer in gel	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)	Buffer based on 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

DIGE Gel is intended for use with the DIGE Buffer Kit and Ettan DALT Large Vertical System. Ettan DALT*twelve* and Ettan DALT*six* instruments are designed for large-format, second dimension 2-D electrophoresis using either 18 or 24 cm Immobiline DryStrip Gels. With Ettan DALT*twelve*, up to 12 DIGE Gels can be run simultaneously with separations complete in 4–6 h. The DIGE Buffer Kit contains the amount of reagents necessary for a single run of up to 12 DIGE Gels using the Ettan DALT*twelve* electrophoresis unit, or two runs of up to six gels using the Ettan DALT*six* unit.

High quality protein spot maps

Homecast polyacrylamide gels give high quality separation and resolution, but the manual step of casting second dimension gels introduces gel-to-gel variation. Figure 2 shows that DIGE Gels provide high sensitivity and high resolution protein spot maps of equal quality compared to homecast polyacrylamide gels.

It is important to note that protein samples run on homecast polyacrylamide gels, DIGE Gel or DALT Gel 12.5 (12.5%) will give rise to differences in spot maps due to varying gel composition and buffer system. DIGE Gel and DALT Gel 12.5 result in very similar protein spot maps, and the DIGE Buffer Kit is compatible with both types of precast gels. In Figure 3, DIGE Gels were run with conventional Laemmli (Tris-Glycine) buffer which can result in protein spot maps distorted in the low molecular weight region (Fig 3A). Figure 3B shows that using the DIGE Buffer Kit, together with DIGE Gels, results in superior protein spot maps with focused and clearly resolved protein spots, even in the low molecular weight region.

Technical replicates were run using either DIGE Gels or homecast polyacrylamide gels. Spot matching was performed, and the number of matched protein spots in the replicate analyses were plotted. Figure 4 shows that DIGE Gel gives spot maps with a higher reproducibility compared to homecast gels. The figure shows that more protein spots were consistently matched from the technical replicates using DIGE Gels with DIGE Buffer Kit.

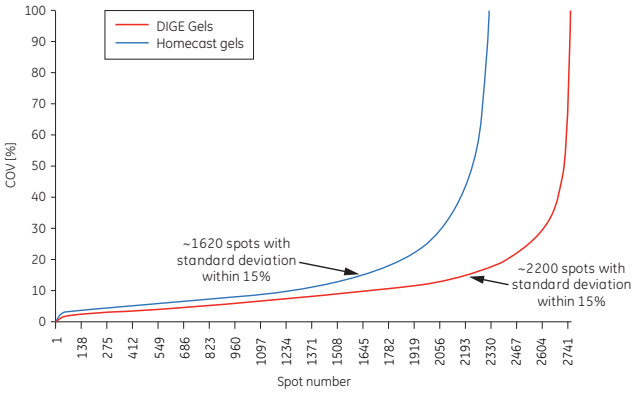

Fig 4. Higher reproducibility with DIGE Gel: DIGE Gels run with DIGE Buffer Kit or homecast polyacrylamide gels run with Laemmli buffer. Eight technical replicates of DIGE Gels, and six technical replicates of homecast polyacrylamide gels. Log abundance for all matched spots exported from DeCyder Biological Variation Analysis (BVA) module, recalculated to covariance (%) and plotted.

References

1. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685 (1970).

Sample labeling:	*E.coli* protein sample (50 µg) labeled with CyDye DIGE Fluor, Cy™5 minimal dye
Sample buffer:	7 M urea, 2 M thiourea, 2% CHAPS, 130 mM DTT, and 2% IPG Buffer pH 4–7.
First dimension IEF:	Immobiline DryStrip pH 4–7, 24 cm rehydrated overnight at room temperature in DeStreak Rehydration Solution + 2% IPG Buffer pH 4–7. Separation using Ettan IPGphor™ 3 IEF unit, anodic cup loading, 60-62 kVh.

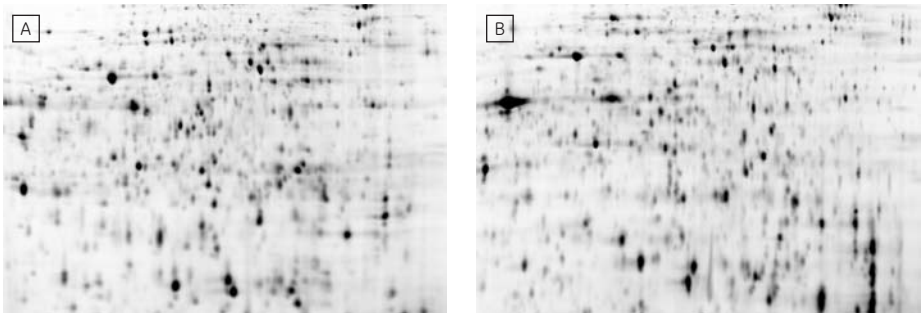

Fig 2. Second dimension separation using Ettan DALT*twelve* with (A) 12.5% homecast polyacrylamide gels or (B) 12.5% precast DIGE Gels. DIGE Gels provide high resolution protein spot maps of equal quality compared to homecast polyacrylamide gels.

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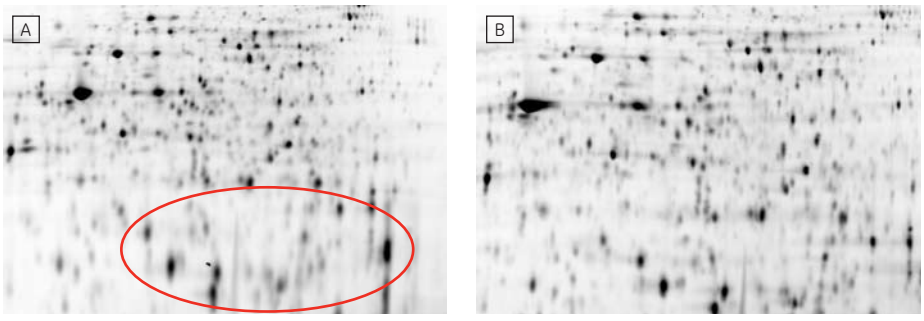

Fig 3. Second dimension separation using 12.5% precast DIGE Gels run on Ettan DALT*twelve* with (A) Tris-Glycine buffer or (B) DIGE Buffer Kit. With conventional Tris-Glycine buffer, protein spot maps can be distorted in the low molecular weight region (red circle). Using DIGE Buffer Kit, together with DIGE Gels, gives clearly resolved spot maps even in the low molecular weight region.

Synchronized solutions for better 2-D electrophoresis - improved 2-D DIGE

2-D electrophoresis is a powerful technology for comparing complex protein mixtures from biological samples in proteomics research. With the aim of simplifying the process and improving quantitation, GE Healthcare has made significant improvements across the entire workflow, from sample prep and separation to data analysis. 2-D DIGE (Difference Gel Electrophoresis) allows you to analyze differences in low abundance proteins with unparalleled statistical confidence. Many of our 2-D electrophoresis products improve data quality from traditional 2-D experiments as well, and the products can eventually be integrated into a complete solution to maximally improve your 2-D results.

Sample Preparation	Protein Labeling	Protein Separation	Image Acquisition	Image Analysis	Validation
2-D Protein Extraction Buffer	DIGE Trial Pack CyDye™ DIGE Fluor 2 nmol kit CyDye DIGE 400 nmol sat dye CyDye DIGE repackaging	IPGbox Immobiline™ DryStrip gels IPG Buffer DIGE Gel and DIGE Buffer Kit	Ettan™ DIGE Imager Typhoon™ Variable Mode Imager	DeCyder™ 2-D software v7.0	ECL Plex™ ImageQuant™ TL 7.0 with ImageQuant TL SecurITy

For more information, visit **www.gelifesciences.com/2DE**

Ordering information

Product	Quantity	Code No
DIGE Gel	3	28-9374-51
DIGE Buffer Kit	2 × 125 ml anode buffer	
	4 × 125 ml cathode buffer	
	12 tubes agarose sealing solution	28-9374-52

Related Products	Quantity	Code No
Sample labeling		
DIGE Trial Pack, incl. CyDye DIGE Fluor minimal dye labeling kit (2 nmol) + 1 month free DeCyder 2-D trial license	1	28-9373-73
CyDye DIGE Fluor minimal dye labeling kit	2 nmol	28-9345-30
CyDye DIGE Fluor minimal dye labeling kit	5 nmol	25-8010-65
CyDye DIGE Fluor Cy2 minimal dye	5 nmol	25-8010-82
CyDye DIGE Fluor Cy3 minimal dye	5 nmol	25-8010-83
CyDye DIGE Fluor Cy5 minimal dye	5 nmol	25-8010-85
CyDye DIGE Fluor Cy2 minimal dye	10 nmol (2 × 5)	25-8008-60
CyDye DIGE Fluor Cy3 minimal dye	10 nmol (2 × 5)	25-8008-61
CyDye DIGE Fluor Cy5 minimal dye	10 nmol (2 × 5)	25-8008-62
CyDye DIGE Fluor Cy2 minimal dye	25 nmol (5 × 5)	RPK0272
CyDye DIGE Fluor Cy2 minimal dye	25 nmol (5 × 5)	RPK0273
CyDye DIGE Fluor Cy2 minimal dye	25 nmol (5 × 5)	RPK0275
CyDye DIGE Fluor Labeling Kit for Scarce Samples	1	25-8009-83
CyDye DIGE Fluor Labeling Kit for Scarce Samples and Preparative Gel Labeling	1	25-8009-84
CyDye DIGE Fluor Preparative Gel Labeling for Scarce Samples	400 nmol	28-9366-83

Related Products	Quantity	Code No
First dimension electrophoresis		
Ettan IPGphor3 Isoelectric Focusing Unit	1	11-0033-64
IPGphor Manifold ceramic tray, complete	1	80-6498-38
IPGbox	1 IPGbox + IPGbox Kit	28-9334-65
IPGbox Kit	10 Reswell Trays + IPGbox Insert	28-9334-92
IPG Buffer pH 3.5-5.0	1 ml	17-6002-02
IPG Buffer pH 5.5-6.7	1 ml	17-6002-06
IPG Buffer pH 4-7	1 ml	17-6000-86
IPG Buffer pH 6-11	1 ml	17-6001-78
IPG Buffer pH 7-11 NL	1 ml	17-6004-39
IPG Buffer pH 3-10 NL	1 ml	17-6000-88
IPG Buffer pH 3-10	1 ml	17-6000-87
IPG Buffer pH 3-11 NL	1 ml	17-6004-40
DeStreak Rehydration Solution	5 × 3 ml	17-6003-19
DeStreak Reagent	1 ml	17-6003-18

Immobiline DryStrip Gels for IEF

pH range	Quantity	Code No 18 cm	Code No 24 cm
3.5–4.5	12/pack	-	17-6002-38
3–7 NL	12/pack	-	17-6002-43
4–7	12/pack	17-1233-01	17-6002-46
6–9	12/pack	17-6001-88	17-6002-47
6–11	12/pack	17-6001-97	-
3–10	12/pack	17-1234-01	17-6002-44
3–10 NL	12/pack	17-1235-01	17-6002-45
3–5.6 NL	12/pack	17-6003-56	17-6003-57
5.3–6.5	12/pack	17-6003-61	17-6003-62
6.2–7.5	12/pack	17-6003-66	17-6003-67
7–11 NL	12/pack	17-6003-71	17-6003-72
3-11 NL	12/pack	17-6003-76	17-6003-77

Related Products	Quantity	Code No
Second dimension electrophoresis		
Ettan DALT*twelve* Separation Unit & Power Supply/Control Unit, 230 V	1	80-6466-27
Ettan DALT*twelve* Separation Unit & Power Supply/Control Unit, 115 V	1	80-6466-46
Ettan DALT*six* Electrophoresis Unit, 220 V	1	80-6485-27
Ettan DALT*six* Electrophoresis Unit, 115 V	1	80-6485-08

Related Products	Quantity	Code No
Image capture and analysis		
Typhoon Trio™ with ImageQuant™ TL and PC	1	63-0055-88
Typhoon Trio+ with ImageQuant TL and PC	1	63-0055-90
Typhoon 9400 with ImageQuant TL and PC	1	63-0055-79
Typhoon 9410 with ImageQuant TL and PC	1	63-0055-81
Ettan DIGE Imager	1	63-0056-42
DeCyder 2-D v7.0 Software Package	1	28-9435-83
DeCyder 2-D v7.0 Oracle 10gRZ, 5 user license	1	28-9435-88
DeCyder 2-D v7.0, 1 user license	1	28-9442-75

Related literature	Code No
2-D Electrophoresis: Principles and Methods, Handbook	80-6429-60
Ettan DIGE System User Manual	18-1173-17

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DIGE Gel and DIGE Buffer Kit: The buffer system in this gel and buffer kit is covered by patent application WO9616724 granted in US, EP and JP.

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