



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

2-D Fluorescence Difference Gel Electrophoresis

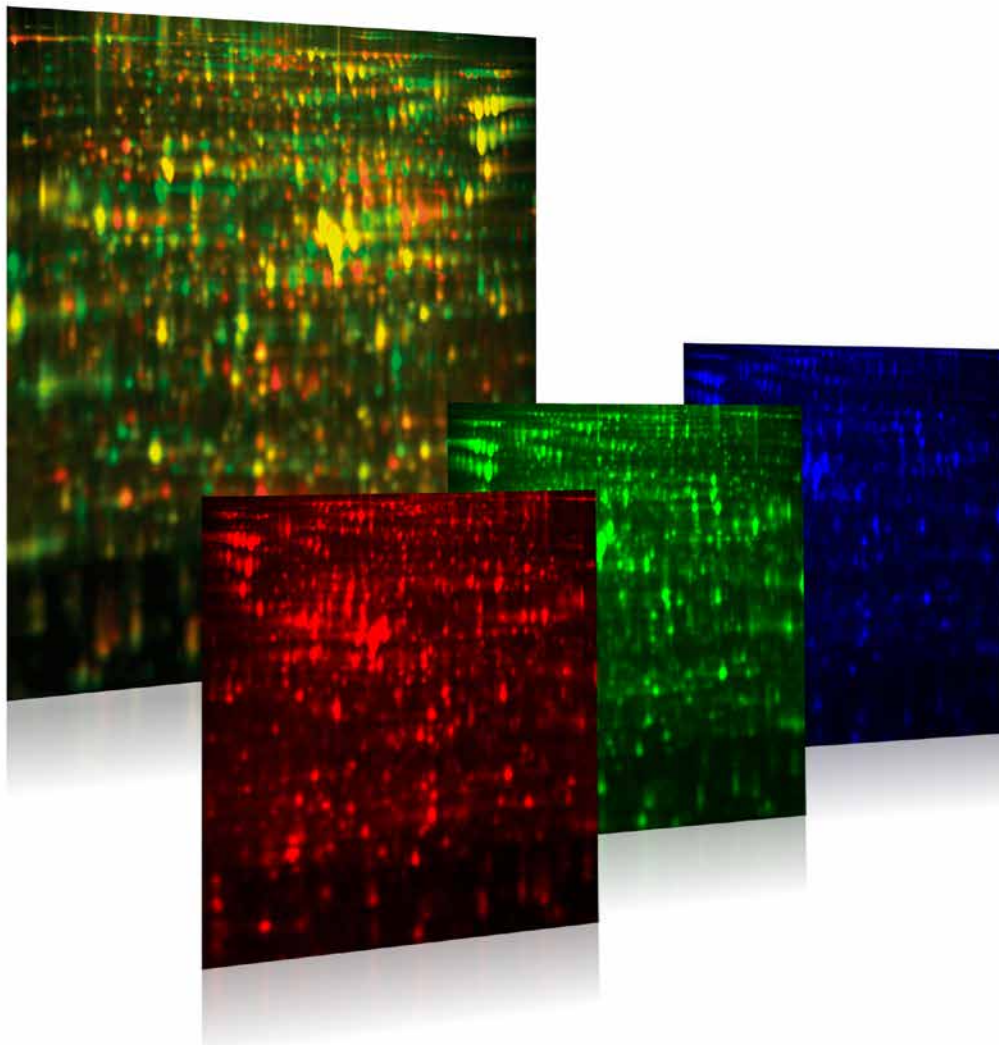


How 2-D DIGE can help you

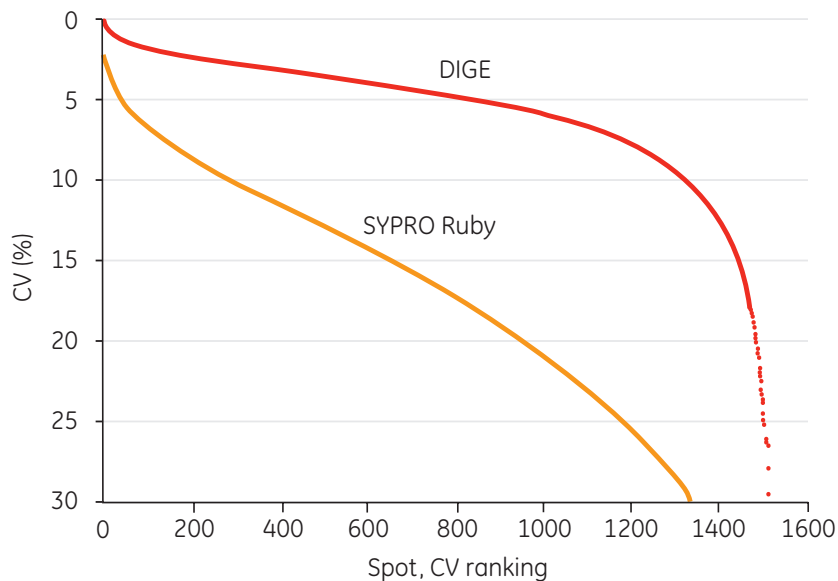
2-D DIGE is a platform for the analysis of differential protein expression. When you want to detect and analyze differences or changes in protein levels/expression between complex protein samples, 2-D DIGE combines confidence with reproducibility to give dependable results.

The Ettan™ DIGE System consists of:

- CyDye™ DIGE fluors for protein labeling
- Imager (Typhoon™ FLA 9000) for image capture
- DeCyder™ 2-D Differential Analysis Software for image analysis



Compared to conventional 2-D electrophoresis, 2-D DIGE delivers:

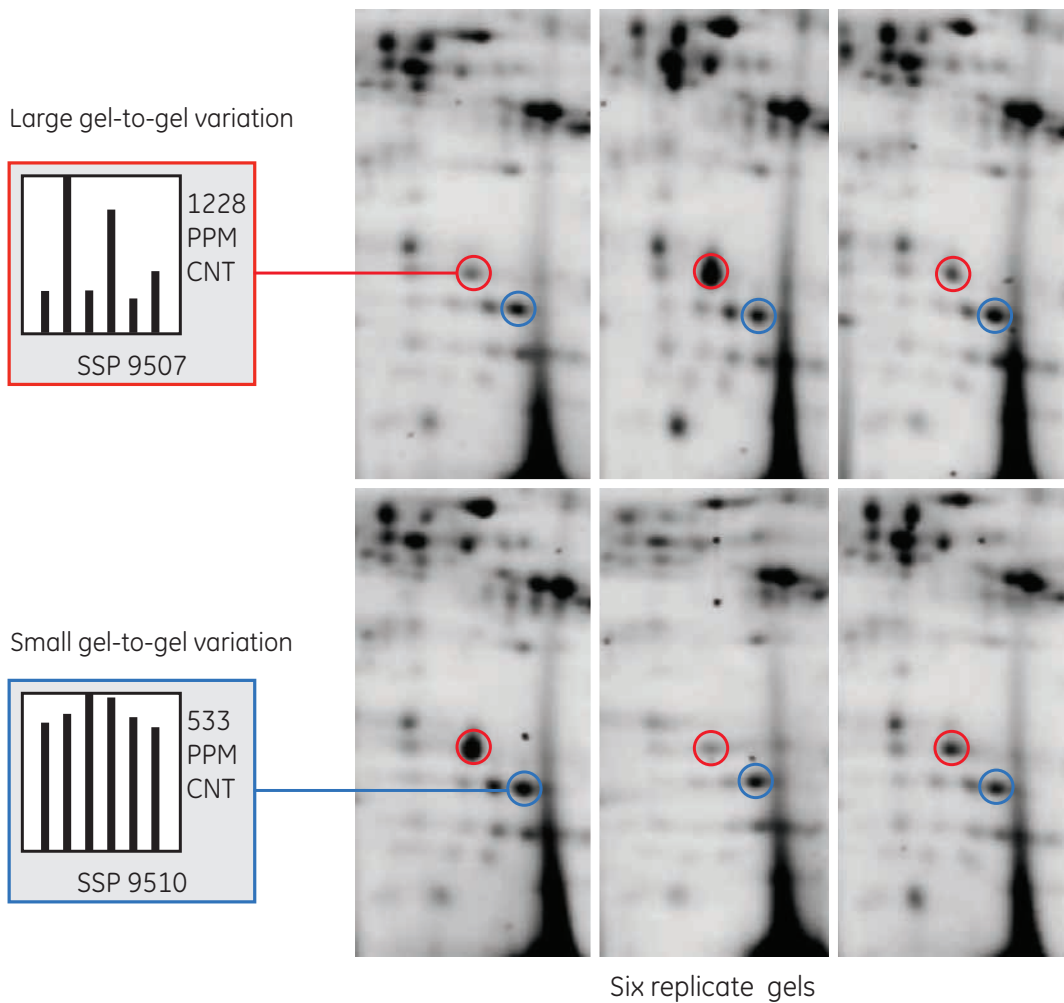


- an internal standard that virtually eliminates experimental gel-to-gel variation. No technical replicates need to be run to confirm differences in protein abundance
- increased throughput and significantly reduced analysis time and cost (pre-labeling of samples instead of post-staining of gels)
- dependable analysis results with far fewer 2-D gels (multiplexing two samples per gel with only biological replicates needed)
- detection of the smallest possible real differences in protein expression with unparalleled statistical confidence

Coefficient of variation (CV) in six replicate 2-D gels of the same sample vs. spot number, ranked by spot CV. Detection of spots by either SYPRO Ruby or CyDye DIGE fluor minimal labeling. Data courtesy of Dr. Jörgen Östling, AstraZeneca R&D Mölndal, Sweden.

Identification of differences in protein expression

Without 2-D DIGE



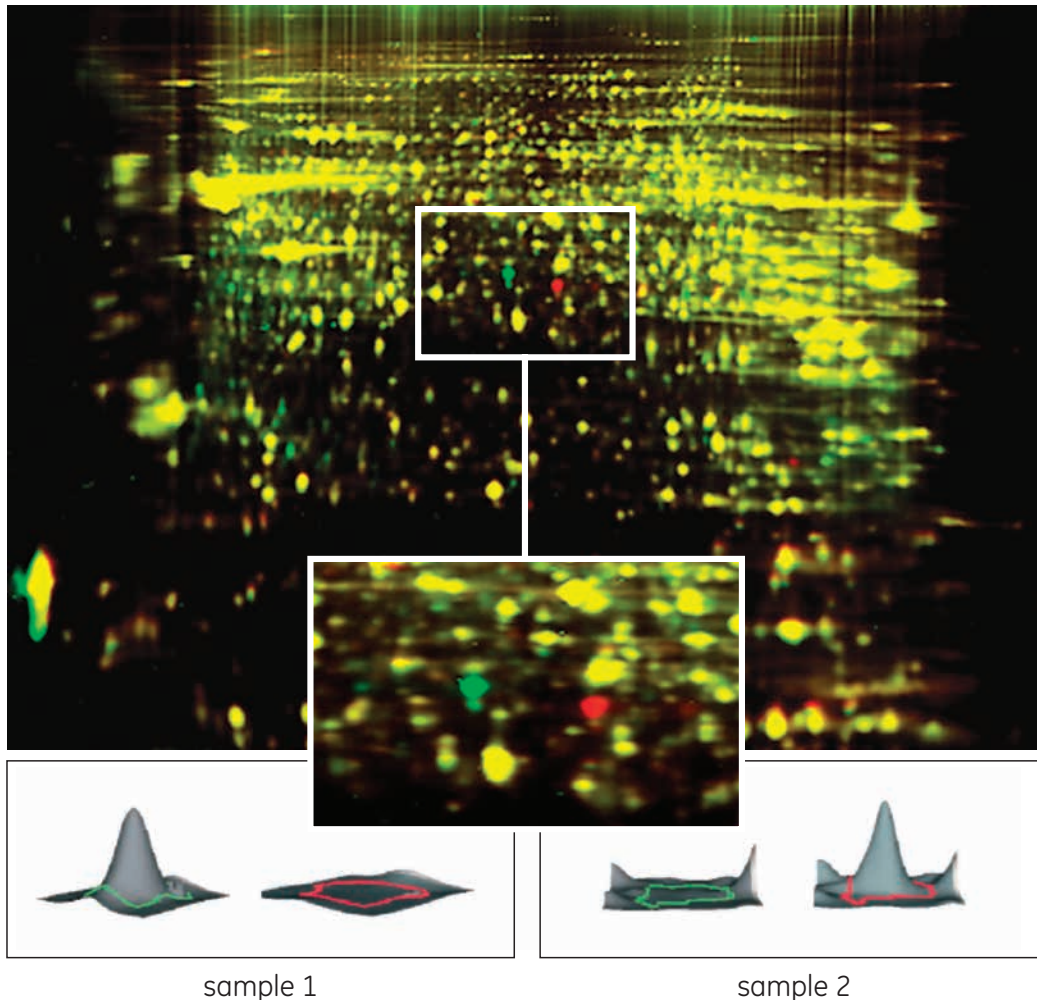
Many technical replicate gels required due to high gel-to-gel variation

Time-consuming post-staining of gels required

Low confidence level on detected differences

Time wasted chasing false positives

Identification of differences in protein expression With 2-D DIGE



Allows detection of up to three pre-labeled protein samples on the same 2-D gel.

Multiplexed analysis enables analysis of multiple samples using the same 2-D electrophoresis conditions on the same 2-D gel, which minimizes experimental variation and ensures accurate within-gel matching.

High confidence level in detected differences.

Identification of true differences.

In sample 1, the left protein spot (green) is expressed, but not the protein spot to the right (red).
In sample 2, the reverse is true.

Protein expression changes in epilepsy-induced rats.
Courtesy of Dr S. Hattori, Division of Cell Proteomics, Institute of Medical Science, University of Tokyo, Japan

Praise for 2-D DIGE

“Without the benefit of the internal standard, 42 of these proteins would have been overlooked due to variation between normal and tumor samples ... compared with individual DIGE comparisons made within a single 2-D gel.”

Friedman, D. B. *et al.* Proteome analysis of human colon cancer by two-dimensional difference gel electrophoresis and mass spectrometry. *Proteomics* **4**, 793–811 (2004).

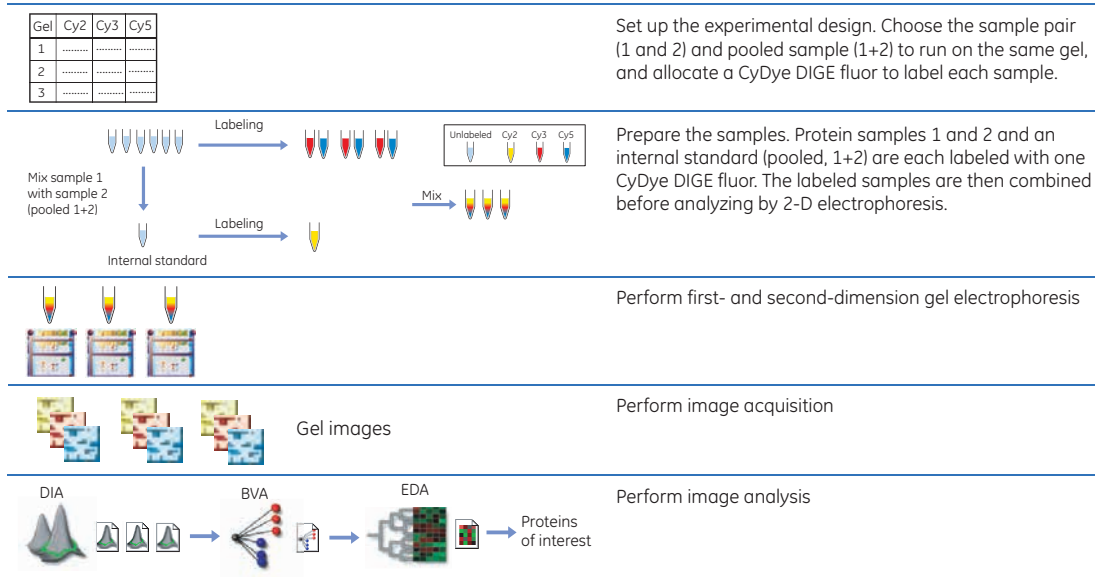


2-D DIGE received the Frost & Sullivan Award for Technology Innovation 2007 with this motivation

“GE’s Ettan DIGE System is capable of comparing protein expression patterns from two different samples in a single gel. This information is crucial in the search for biomarkers that may change in expression levels during the initiation or progression of a disease from one phenotype to a more malignant phenotype.

The need to isolate and identify these protein biomarkers that appear or fail to appear is likely to also influence the way patients’ treatment protocols are determined.”

Overview of the 2-D DIGE analytical workflow



The subsequent goal is to identify the protein(s) of interest through protein spot picking, digestion, and mass spectrometry. 2-D DIGE ensures that real differences between the samples are identified.

For further information, see *Ettan DIGE System User Manual*
See also the *Protein and nucleic acid sample preparation Selection Guide*

Sample preparation for 2-D DIGE

Sample preparation	Code no.	Description
2-D Protein Extraction Buffer Trial Kit	45002168	Contains each of the six extraction buffers, to screen for the most appropriate extraction buffer for your sample
2-D Protein Extraction Buffer-I	45002167	Ready-to-mix buffer for efficient cell lysis and protein extraction. Note: This buffer is not compatible with CyDye DIGE minimal dyes
2-D Protein Extraction Buffer-II	45002166	Ready-to-mix buffer for efficient cell lysis and protein extraction
2-D Protein Extraction Buffer-III	45002165	Ready-to-mix buffer for efficient cell lysis and protein extraction. Note: This buffer is not compatible with CyDye DIGE saturation dyes
2-D Protein Extraction Buffer-IV	45002164	Ready-to-mix buffer for efficient cell lysis and protein extraction. Note: This buffer is not compatible with CyDye DIGE saturation dyes
2-D Protein Extraction Buffer-V	45002165	Ready-to-mix buffer for efficient cell lysis and protein extraction
2-D Protein Extraction Buffer-VI	45002162	Ready-to-mix buffer for efficient cell lysis and protein extraction
Sample Grinding Kit	45001731	The mechanical breaking up of cells or tissue releasing the total protein content
2-D Clean-Up Kit	45001737	Removal of interfering contaminants and concentration of total protein
Protease Inhibitor Mix	45001740	Protease inhibitor cocktail
Nuclease Mix	45001741	Cocktail of nucleases for the removal of DNA and RNA from the protein sample
Albumin and IgG Removal Kit	45002176	Removal of albumin and IgG from a human serum sample, spin format
2-D Quant Kit	45001732	Quantitation of the protein amount in denaturing buffers
Tris	45000236	Suitable for preparing electrophoresis buffer in the pH range 7.2 to 9.0
Urea	45000234	Solubilizes and unfolds most proteins to their fully random conformation
Thiourea	45001296	Chaotrope, used together with urea in the IPG strip rehydration solution
CHAPS	45000228	A zwitterionic detergent commonly used in IEF
Iodoacetamide	45001297	For efficient alkylation of thiols
SDS	45000227	Ionic detergent used to solubilize proteins
PlusOne DTT	45000232	Reducing agent included in the sample solution to break disulfide bonds and maintain all proteins in their fully reduced state
illustra triplePrep Kit	45001810	Isolate gDNA, total RNA, and total denatured proteins from undivided tissue and cell samples
Vivaspin Sample Concentrators	various	For sample concentration. For details, please refer to Data file 28-9356-53

CyDye labeling

CyDye fluors, see page 9		
Dimethylformamide (DMF)		Anhydrous DMF; should be replaced with fresh bottle every 3 months
L-cysteine (only minimal)		Only when minimal labeling is performed. Quenches the minimal labeling reaction
TCEP Tris-2-carboxyethyl phosphine hydrochloride (only saturation)		Only when saturation labeling is performed. Reduces disulfide bonds prior to saturation labeling on cysteines

CyDye fluors for 2-D DIGE

CyDye DIGE fluor labeling	Code no.	Description
CyDye DIGE fluor minimal dyes		Minimal labeling procedure
CyDye DIGE Kit, 2 nmol	45002245	For initial trial or scouting studies. All three dyes (Cy [™] 2, Cy3, and Cy5) in one kit Sufficient for five labelings with each dye
CyDye DIGE Fluor, minimal labeling kit, 5 nmol	45002256	All three dyes in one kit. Enough for a small study with up to 12 labelings with each dye
CyDye DIGE Cy2, 5 nmol	45002259	Enough for a small study with up to 12 labelings
CyDye DIGE Cy3, 5 nmol	45002258	Enough for a small study with up to 12 labelings
CyDye DIGE Cy5, 5 nmol	45002257	Enough for a small study with up to 12 labelings
CyDye DIGE Cy2, 10 nmol	45002253	For experiments with a limited number of variables, up to 25 labelings. Repackaged in 2 × 5 nmol vials
CyDye DIGE Cy3, 10 nmol	45002252	For experiments with a limited number of variables, up to 25 labelings. Repackaged in 2 × 5 nmol vials
CyDye DIGE Cy5, 10 nmol	45002251	For experiments with a limited number of variables, up to 25 labelings. Repackaged in 2 × 5 nmol vials
CyDye DIGE Cy2, 25 nmol	45002244	Suitable for larger experimental set-ups with several variables, up to 60 labelings. Repackaged in 5 × 5 nmol vials
CyDye DIGE Cy3, 25 nmol	45002243	Suitable for larger experimental set-ups with several variables, up to 60 labelings. Repackaged in 5 × 5 nmol vials
CyDye DIGE Cy5, 25 nmol	45002242	Suitable for larger experimental set-ups with several variables, up to 60 labelings. Repackaged in 5 × 5 nmol vials
CyDye DIGE fluor saturation dyes		For saturated labeling of small sample amounts or precious samples
CyDye DIGE Fluor Labeling Kit for Scarce Samples	45002248	For labeling of up to 25 reactions (5 µg protein each). Two dyes in one kit (100 nmol Cy3 sat dye and 100 nmol Cy5 sat dye)
CyDye DIGE Fluor Labeling Kit for Scarce Samples and Preparative Gel Labeling	45002255	For labeling of 25 analytical reactions and preparative labeling of up to 500 µg protein (100 nmol Cy3 sat dye, 100 nmol Cy5 sat dye, and 400 nmol Cy3 sat dye)
CyDye DIGE Fluor Preparative Gel Labeling for Scarce Samples	45002247	For preparative labeling of up to 500 µg protein (400 nmol Cy3 sat dye)

First-dimension isoelectric focusing (IEF)



Ettan IPGphor™ 3



IPGbox, IPGbox Kit, Immobiline™ DryStrip gels (precast IPG strip gels), and IPG Buffers

First-dimension isoelectric focusing (IEF)	Code no.	Description
Ettan IPGphor 3 Isoelectric Focusing Unit	45002623	Unit to run first dimension (IEF)
IPGphor Manifold, Complete	45002613	For the most stringent applications. Exceptionally uniform thermal characteristics.
IPGphor Manifold Light	45002621	Routine applications. Easy to handle and extremely robust.
Immobiline DryStrip gels, 24 cm IMPROVED quality	various	Precast gel for first-dimension IEF
Immobiline DryStrip gels, 18 cm IMPROVED quality	various	Precast gel for first-dimension IEF
Immobiline DryStrip gels, 13 cm IMPROVED quality	various	Precast gel for first-dimension IEF
Immobiline DryStrip gels, 11 cm IMPROVED quality	various	Precast gel for first-dimension IEF
Immobiline DryStrip gels, 7 cm IMPROVED quality	various	Precast gel for first-dimension IEF
IPG Buffers, including IMPROVED IPG Buffer pH 3-10 and pH 3-10 NL	various	Used with Immobiline DryStrip gels to improve protein solubility
IPGbox	45002631	Rehydrates up to 12 Immobiline DryStrip gels
IPGbox Kit	45002627	Contains 10 disposable Reswell Trays and one disposable Insert
DeStreak Rehydration Solution	45000381	Improves reproducibility and quality of 2-D gels, and helps prevent streaking. Recommended for IPG strips with a pH above 7, when cup loaded
DeStreak Reagent	45000380	Add DeStreak Reagent to your own rehydration buffer

See also the Improved Immobiline™ DryStrip gels and IPG Buffer Data file

Second-dimension electrophoresis



DALTtwelve Gel Caster



Ettan DALTsix Electrophoresis Unit



Ettan DALTtwelve Electrophoresis Unit



DIGE Gel and DIGE Buffer Kit

Second-dimension SDS-PAGE	Code no.	Description
DIGE Gel	45002178	Precast polyacrylamide gel in a low fluorescent glass cassette
DIGE Buffer Kit	45002177	Specially designed two-buffer system for running up to twelve DIGE Gels simultaneously
Ettan DALTsix Electrophoresis Unit, 115V	45002611	Runs up to six gels simultaneously, enabling separation of 12 DIGE samples in one run. 115V model
EPS 601 Power Supply	45000466	Power supply for DALTsix Electrophoresis Unit
MultiTemp™ IV Thermostatic Circulator, 115V	45002496	A temperature control unit for external, closed electrophoresis equipment, 115V
DALTsix Gel Caster, complete	45005916	For casting up to six gels simultaneously
Equilibration tubes (quantity=12)	45002610	For equilibrating IPG strips prior to second dimension
PlusOne Acrylamide IEF 40% Solution	45000216	Convenient solution, virtually eliminating the risk of acrylamide dust in the air
PlusOne N,N'- Methylenebisacrylamide	45000221	General cross-linker used with acrylamide for polyacrylamide gels
ReadySol IEF 40% T, 3% C	45000224	Ensures a reproducible solution composition (includes cross-linker)
PlusOne TEMED	45000226	Catalyst for the polymerization of acrylamide when used with ammonium persulfate
PlusOne Ammonium Persulfate	45000225	Initiator for polymerization of acrylamide
PlusOne Glycine	45000238	Frequently used in electrophoresis buffers
PlusOne Glycerol	45000240	For displacing and equilibration solutions
PlusOne Bind-silane	45000243	Used to covalently attach polyacrylamide gels to a glass surface

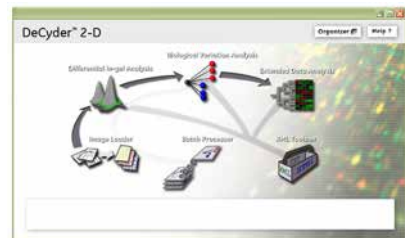
Validation

Protein labeling and detection	Code no.	Description
ECL Plex Western Blotting Combination Pack (Cy3, Cy5, Hybond ECL)	45001214	ECL Plex fluorescently labeled: goat- α -mouse IgG-Cy3, goat- α -rabbit IgG-Cy5, and Rainbow Markers. Hybond ECL, 10 x 10 cm, 10 sheets and protocol. Sufficient for at least 1000 cm ² of membrane
ECL Plex Western Blotting Combination Pack (Cy3, Cy5, Hybond-LFP)	45001215	ECL Plex fluorescently labeled: goat- α -mouse IgG-Cy3, goat- α -rabbit IgG-Cy5, and Rainbow Markers. Hybond-LFP, 20 x 20 cm, 3 sheets and protocol. Sufficient for at least 1000 cm ² of membrane
ECL Plex Fluorescent Rainbow Markers, 120 μ l	45001594	Protein molecular weight markers optimized for use with ECL Plex Western Blotting Detection System. Sufficient for 50 samples
ECL Plex Fluorescent Rainbow Markers, 500 μ l	45001595	Protein molecular weight markers optimized for use with ECL Plex Western Blotting Detection System. Sufficient for 200 samples

Also available from GE Healthcare to enhance your 2D DIGE system:



Typhoon FLA 9000



DeCyder 2-D



Ettan Spot Picker



Ettan Digester

Ask us for more information
www.gelifesciences.com/2DE

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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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