



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

2-D Fluorescence Difference Gel Electrophoresis

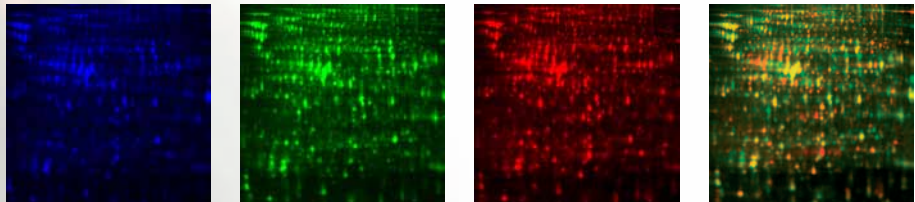


# How 2-D DIGE can help you

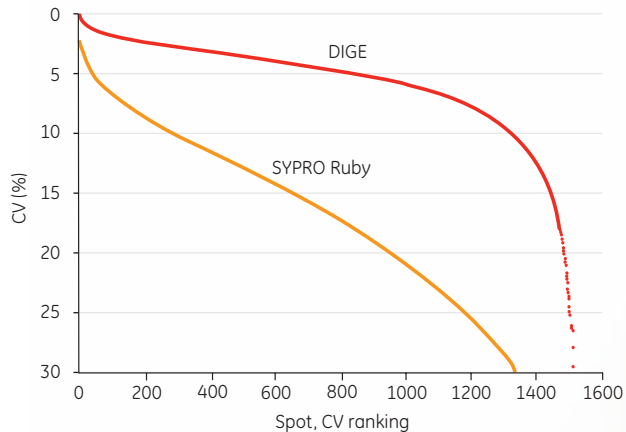
2-D DIGE is a platform for the analysis of differential protein expression. When you want to detect and analyze differences or changes in protein levels/expression between complex protein samples, 2-D DIGE combines confidence with reproducibility to give dependable results.

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:

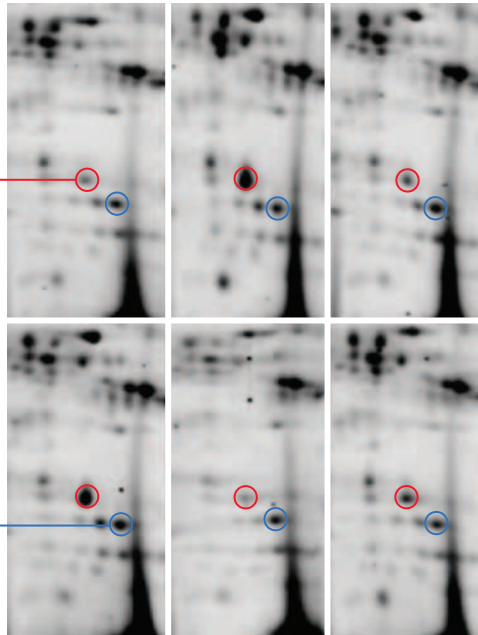
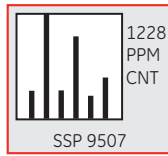


*Coefficient of variation (CV) in six replicate 2-D gels of the same sample vs. spot number, ranked by spot CV. Detection of spots by either SYPRO Ruby or CyDye DIGE fluor minimal labeling. Data courtesy of Dr. Jörgen Östling, AstraZeneca R&D Mölndal, Sweden.*

- 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

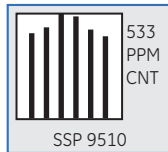
# Identification of differences in protein expression Without 2-D DIGE

Large gel-to-gel  
variation



Six replicate gels

Small gel-to-gel  
variation



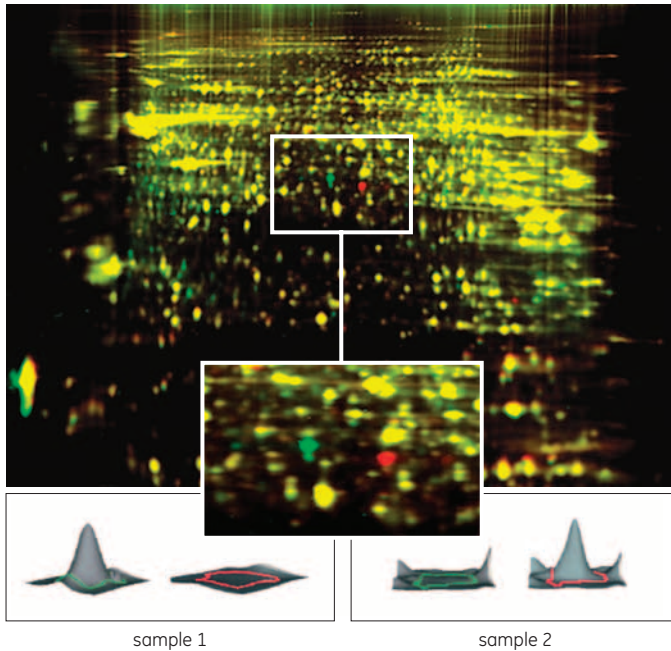
Many technical replicate  
gels required due to high  
gel-to-gel variation

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."*



# Sample preparation for 2-D DIGE

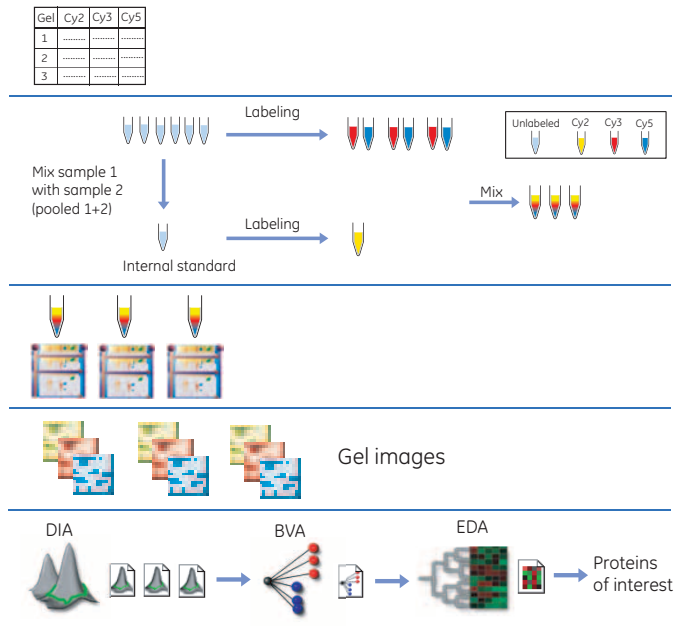
Sample preparation	Code no.	Description
2-D Protein Extraction Buffer Trial Kit	28-9434-22	Contains each of the six extraction buffers, to screen for the most appropriate extraction buffer for your sample
2-D Protein Extraction Buffer-I	28-9434-23	Ready-to-mix buffer for efficient cell lysis and protein extraction. Note: This buffer is not compatible with CyDye DIGE minimal dyes
2-D Protein Extraction Buffer-II	28-9434-24	Ready-to-mix buffer for efficient cell lysis and protein extraction
2-D Protein Extraction Buffer-III	28-9434-25	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	28-9434-26	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	28-9434-27	Ready-to-mix buffer for efficient cell lysis and protein extraction
2-D Protein Extraction Buffer-VI	28-9434-28	Ready-to-mix buffer for efficient cell lysis and protein extraction
Sample Grinding Kit	80-6483-37	The mechanical breaking up of cells or tissue releasing the total protein content
2-D Clean-Up Kit	80-6484-51	Removal of interfering contaminants and concentration of total protein
Protease Inhibitor Mix	80-6501-23	Protease inhibitor cocktail
Nuclease Mix	80-6501-42	Cocktail of nucleases for the removal of DNA and RNA from the protein sample
Albumin and IgG Removal Kit	RPN6300	Removal of albumin and IgG from a human serum sample, spin format
2-D Quant Kit	80-6483-56	Quantitation of the protein amount in denaturing buffers
Tris	17-1321-01	Suitable for preparing electrophoresis buffer in the pH range 7.2 to 9.0
Urea	17-1319-01	Solubilizes and unfolds most proteins to their fully random conformation
Thiourea	RPN6301	Chaotrope, used together with urea in the IPG strip rehydration solution
CHAPS	17-1314-01	A zwitterionic detergent commonly used in IEF
Iodoacetamide	RPN6302	For efficient alkylation of thiols
SDS	17-1313-01	Ionic detergent used to solubilize proteins
PlusOne DTT	17-1318-01	Reducing agent included in the sample solution to break disulfide bonds and maintain all proteins in their fully reduced state
illustra triplePrep Kit	28-9425-44	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)	Sigma Aldrich 22,705-6	Anhydrous DMF; should be replaced with fresh bottle every 3 months
L-lysine (only minimal)	Sigma L5626	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

See also the *Protein and nucleic acid sample preparation Selection Guide*, 28-9337-00

# Overview of the 2-D DIGE analytical workflow



Set up the experimental design. Choose the sample pair (1 and 2) and pooled sample (1+2) to run on the same gel, and allocate a CyDye DIGE fluor to label each sample.

Prepare the samples. Protein samples 1 and 2 and an internal standard (pooled, 1+2) are each labeled with one CyDye DIGE fluor. The labeled samples are then combined before analyzing by 2-D electrophoresis.

Perform first- and second-dimension gel electrophoresis

Perform image acquisition

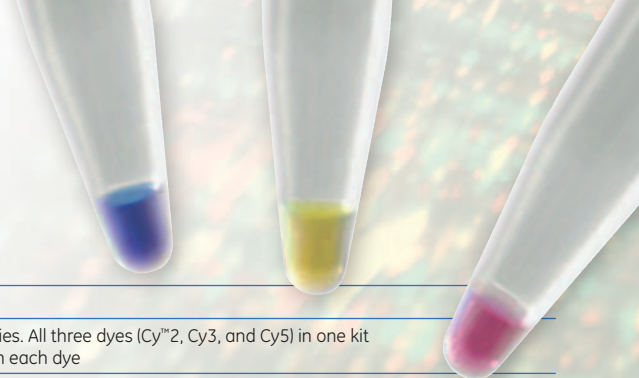
Perform image analysis

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*, 18-1173-17



# CyDye fluors for 2-D DIGE



<b>CyDye DIGE fluor labeling</b>	<b>Code no.</b>	<b>Description</b>
<b>CyDye DIGE fluor minimal dyes</b>		<b>Minimal labeling procedure</b>
CyDye DIGE Kit, 2 nmol	28-9345-30	For initial trial or scouting studies. All three dyes (Cy <sup>™</sup> 2, Cy3, and Cy5) in one kit Sufficient for five labelings with each dye
CyDye DIGE Fluor, minimal labeling kit, 5 nmol	25-8010-65	All three dyes in one kit. Enough for a small study with up to 12 labelings with each dye
CyDye DIGE Cy2, 5 nmol	25-8010-82	Enough for a small study with up to 12 labelings
CyDye DIGE Cy3, 5 nmol	25-8010-83	Enough for a small study with up to 12 labelings
CyDye DIGE Cy5, 5 nmol	25-8010-85	Enough for a small study with up to 12 labelings
CyDye DIGE Cy2, 10 nmol	25-8008-60	For experiments with a limited number of variables, up to 25 labelings. Repackaged in 2 × 5 nmol vials
CyDye DIGE Cy3, 10 nmol	25-8008-61	For experiments with a limited number of variables, up to 25 labelings. Repackaged in 2 × 5 nmol vials
CyDye DIGE Cy5, 10 nmol	25-8008-62	For experiments with a limited number of variables, up to 25 labelings. Repackaged in 2 × 5 nmol vials
CyDye DIGE Cy2, 25 nmol	RPK0272	Suitable for larger experimental set-ups with several variables, up to 60 labelings. Repackaged in 5 × 5 nmol vials
CyDye DIGE Cy3, 25 nmol	RPK0273	Suitable for larger experimental set-ups with several variables, up to 60 labelings. Repackaged in 5 × 5 nmol vials
CyDye DIGE Cy5, 25 nmol	RPK0275	Suitable for larger experimental set-ups with several variables, up to 60 labelings. Repackaged in 5 × 5 nmol vials
<b>CyDye DIGE fluor saturation dyes</b>		<b>For saturated labeling of small sample amounts or precious samples</b>
CyDye DIGE Fluor Labeling Kit for Scarce Samples	25-8009-83	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	25-8009-84	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	28-9366-83	For preparative labeling of up to 500 µg protein (400 nmol Cy3 sat dye)

# First-dimension isoelectric focusing (IEF)



Etan 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	11-0033-64	Unit to run first dimension (IEF)
IPGphor Manifold, Complete	80-6498-38	For the most stringent applications. Exceptionally uniform thermal characteristics.
IPGphor Manifold Light	11-0026-88	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	28-9334-65	Rehydrates up to 12 Immobiline DryStrip gels
IPGbox Kit	28-9334-92	Contains 10 disposable Reswell Trays and one disposable Insert
DeStreak Rehydration Solution	17-6003-19	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	17-6003-18	Add DeStreak Reagent to your own rehydration buffer

See also the *Improved Immobiline™ DryStrip gels and IPG Buffer Data file, 28-9488-45*

# Second-dimension electrophoresis



DALTwelve Gel Caster



Ettan DALSix Electrophoresis Unit



Ettan DALTwelve Electrophoresis Unit



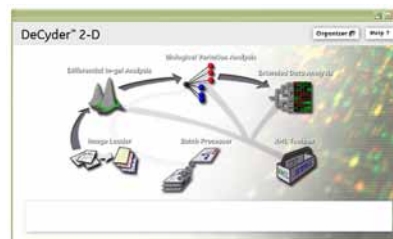
DIGE Gel and DIGE Buffer Kit

<b>Second-dimension SDS-PAGE</b>	<b>Code no.</b>	<b>Description</b>
DIGE Gel	28-9374-51	Precast polyacrylamide gel in a low fluorescent glass cassette
DIGE Buffer Kit	28-9374-52	Specially designed two-buffer system for running up to twelve DIGE Gels simultaneously
Low-fluorescence Glass Plates, 27 × 21 cm (including spacers)	80-6475-58	Suitable for DIGE and other fluorescent samples
Ettan DALTwelve Separation Unit and Power Supply/Control Unit, 115V	80-6466-46	Runs up to 12 gels simultaneously, enabling separation of 24 DIGE samples in one run. 115V model
Ettan DALTwelve Separation Unit and Power Supply/Control Unit, 230V	80-6466-27	Runs up to 12 gels simultaneously, enabling separation of 24 DIGE samples in one run. 230V model
DALTwelve Gel Caster, complete	80-6467-22	For casting up to 14 gels simultaneously
Ettan DALTsix Electrophoresis Unit, 115V	80-6485-08	Runs up to six gels simultaneously, enabling separation of 12 DIGE samples in one run. 115V model
Ettan DALTsix Electrophoresis Unit, 220V	80-6485-27	Runs up to six gels simultaneously, enabling separation of 12 DIGE samples in one run. 220V model
EPS 601 Power Supply	18-1130-02	Power supply for DALTsix Electrophoresis Unit
MultiTemp™ III Thermostatic Circulator, 115V	18-1102-77	A temperature control unit for external, closed electrophoresis equipment, 115V
MultiTemp III Thermostatic Circulator, 230V	18-1102-78	A temperature control unit for external, closed electrophoresis equipment, 230V
DALTsix Gel Caster, complete	80-6485-46	For casting up to six gels simultaneously
Equilibration tubes (quantity=12)	80-6467-79	For equilibrating IPG strips prior to second dimension
PlusOne Acrylamide IEF 40% Solution	17-1301-01	Convenient solution, virtually eliminating the risk of acrylamide dust in the air
PlusOne N,N'- Methylenebisacrylamide	17-1304-02	General cross-linker used with acrylamide for polyacrylamide gels
ReadySol IEF 40% T, 3% C	17-1310-01	Ensures a reproducible solution composition (includes cross-linker)
PlusOne TEMED	17-1312-01	Catalyst for the polymerization of acrylamide when used with ammonium persulfate
PlusOne Ammonium Persulfate	17-1311-01	Initiator for polymerization of acrylamide
PlusOne Glycine	17-1323-01	Frequently used in electrophoresis buffers
PlusOne Glycerol	17-1325-01	For displacing and equilibration solutions

# Imaging and analysis of 2-D DIGE gels



Typhoon FLA 9000



DeCyder 2-D

## Image acquisition

	Code no.	Description
Typhoon FLA 9000	28-9558-08	Three-color (red 635 nm, green 532 nm, and blue 473 nm) laser lines for excitation. Scanner for fluorescent applications and autoradiography. 10 $\mu$ m spatial resolution.

## Image analysis

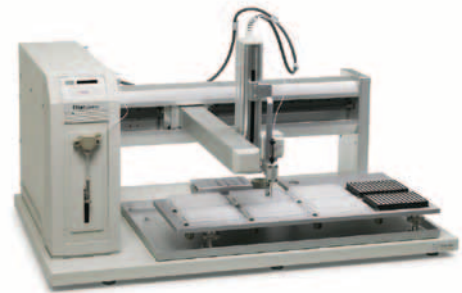
DeCyder 2D 7.2 Preinstalled computer 1-User license	28-9856-57	Dedicated software for analysis of 2-D DIGE data, including extended data analysis and warping. Without SNP.
DeCyder 2D 7.2 Concurrent network license	28-9854-18	One DeCyder 2D 7.2 concurrent network license. Without SNP.
DeCyder 2D 7.2 SPN 1 Concurrent network license	28-9854-11	One DeCyder 2D 7.2 SPN concurrent network license. Dedicated software for full analysis of 2-D DIGE data with a new normalization algorithm (SPN, Spike Protein Normalization), including extended data analysis and warping.
DeCyder 2D 7.2 SPN 1 Concurrent network license Upgrade	28-9854-17	One DeCyder 2D 7.2 SPN concurrent network license Upgrade from DeCyder 2D 7.2
DeCyder 2D Oracle 11g 5 user licenses	28-9435-88	Oracle Database license for five network users



# Products for preparative work



Ettan Spot Picker



Ettan Digester

Protein handling	Code no.	Description
Ettan Spot Picker	18-1145-28	Automatically picks selected protein spots from fluorescently labeled or stained gels
Ettan Digester	18-1142-68	Automatic in-gel digestion of proteins from 2-D gel plugs

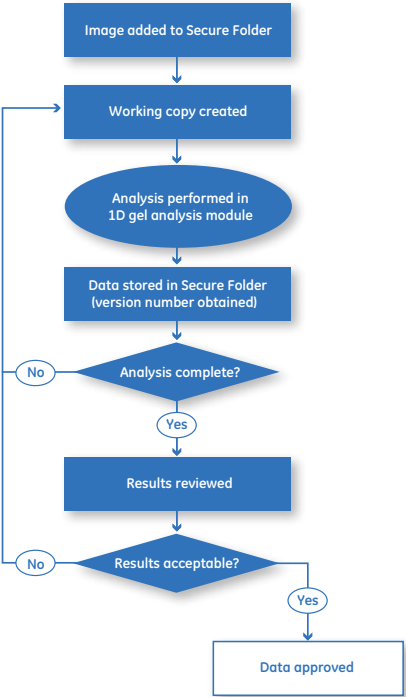
  

Post staining	Code no.	Description
Deep Purple™ Total Protein Stain	RPN6306	Rapid, ultra sensitive fluorescent total protein stain

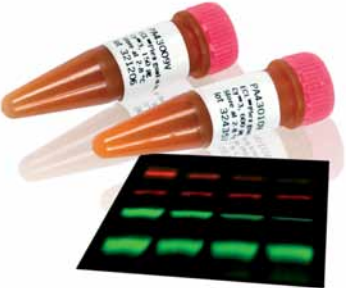
  

Second-dimension SDS-PAGE	Code no.	Description
Reference markers	18-1143-34	White stickers for attaching to low-fluorescent glass plates to enable accurate spot picking. Two sheets, 560 markers/sheet
PlusOne Bind-silane	17-1330-01	Used to covalently attach polyacrylamide gels to a glass surface

# Products for validation



ImageQuant Security



ECL Plex™



ImageQuant TL

<b>Protein labeling and detection</b>	<b>Code no.</b>	<b>Description</b>
ECL Plex Western Blotting Combination Pack (Cy3, Cy5, Hybond ECL)	RPN998	ECL Plex fluorescently labeled: goat- -mouse IgG-Cy3, goat- -rabbit IgG-Cy5, and Rainbow Markers. Hybond ECL, 10 × 10 cm, 10 sheets and protocol. Sufficient for at least 1000 cm <sup>2</sup> of membrane
ECL Plex Western Blotting Combination Pack (Cy3, Cy5, Hybond-LFP)	RPN999	ECL Plex fluorescently labeled: goat- -mouse IgG-Cy3, goat- -rabbit IgG-Cy5, and Rainbow Markers. Hybond-LFP, 20 × 20 cm, 3 sheets and protocol. Sufficient for at least 1000 cm <sup>2</sup> of membrane
ECL Plex Fluorescent Rainbow Markers, 120 µl	RPN850E	Protein molecular weight markers optimized for use with ECL Plex Western Blotting Detection System. Sufficient for 50 samples
ECL Plex Fluorescent Rainbow Markers, 500 µl	RPN851E	Protein molecular weight markers optimized for use with ECL Plex Western Blotting Detection System. Sufficient for 200 samples

<b>Image analysis of 1-D gels and blots</b>	<b>Code no.</b>	<b>Description</b>
ImageQuant TL SecurITy Software package (with CD + Getting started)	28-9380-94	Installation software only, does not include a license. Includes ImageQuant TL as standard. 1-D gel and blot analysis in an enhanced data security environment. Allows full data control and traceability
ImageQuant TL SecurITy Single User License	28-9332-73	License for one user of ImageQuant TL SecurITy
ImageQuant TL SecurITy Site License, 1 additional license	28-9377-35	License for one additional user of ImageQuant TL SecurITy
Upgrade from ImageQuant TL to ImageQuant TL SecurITy, 1-User License	28-9377-80	Upgrade an existing ImageQuant TL license to an ImageQuant TL SecurITy license
ImageQuant TL 1-User License 7.0	28-9236-62	Routine 1-D gel and blot quantitative analysis. License for one user of ImageQuant TL
ImageQuant TL 5-User License 7.0	28-9206-39	License for 5 users of ImageQuant TL
ImageQuant TL 10-User License 7.0	28-9236-57	License for 10 users of ImageQuant TL

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[www.gelifesciences.com/contact](http://www.gelifesciences.com/contact)

GE Healthcare Bio-Sciences AB  
Björkgatan 30  
751 84 Uppsala  
Sweden

[www.gelifesciences.com/2DE](http://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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GE Healthcare UK Limited  
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Europe, GmbH  
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare Bio-Sciences Corp.  
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Japan Corporation  
Sanken Bldg., 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan