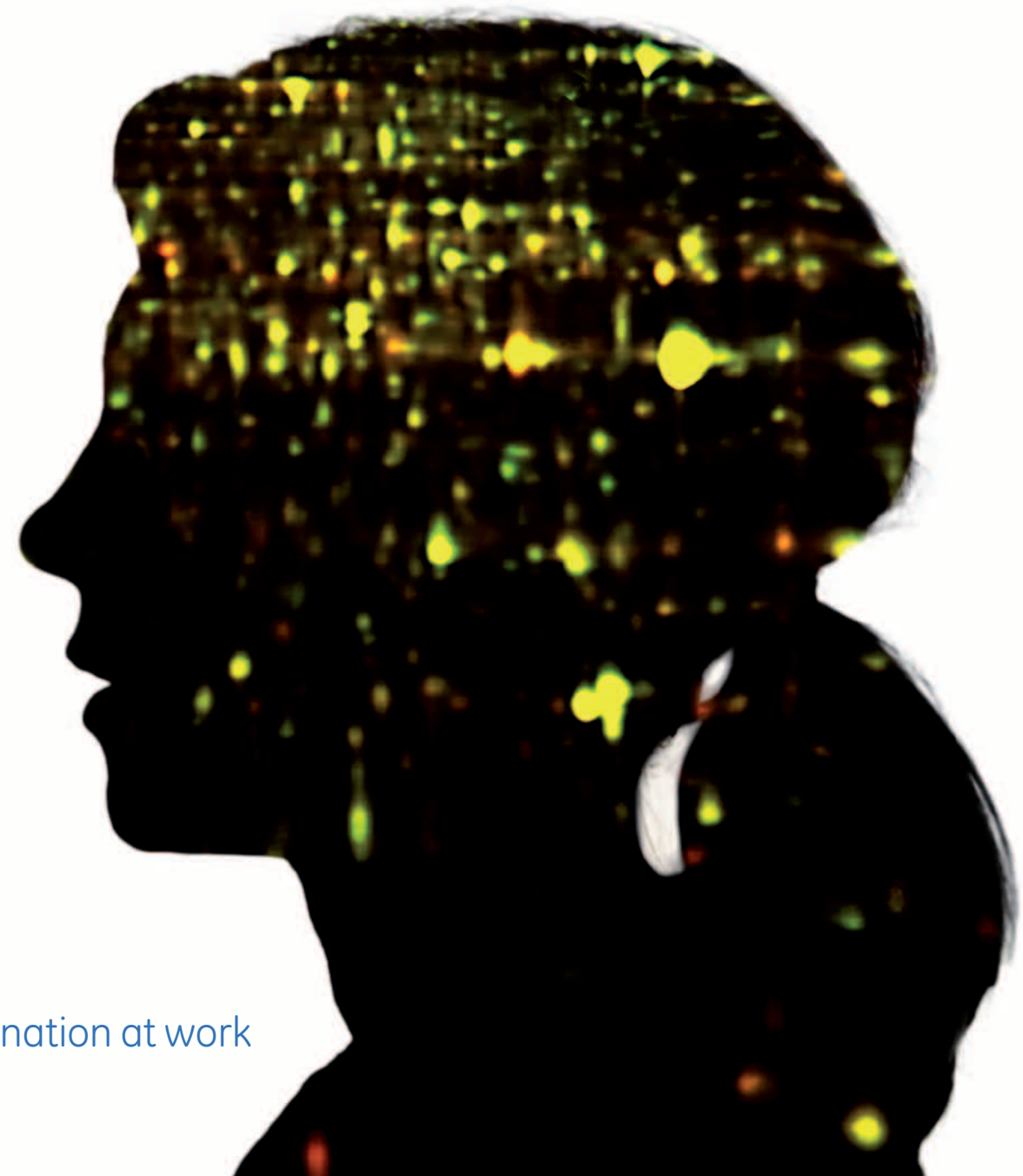
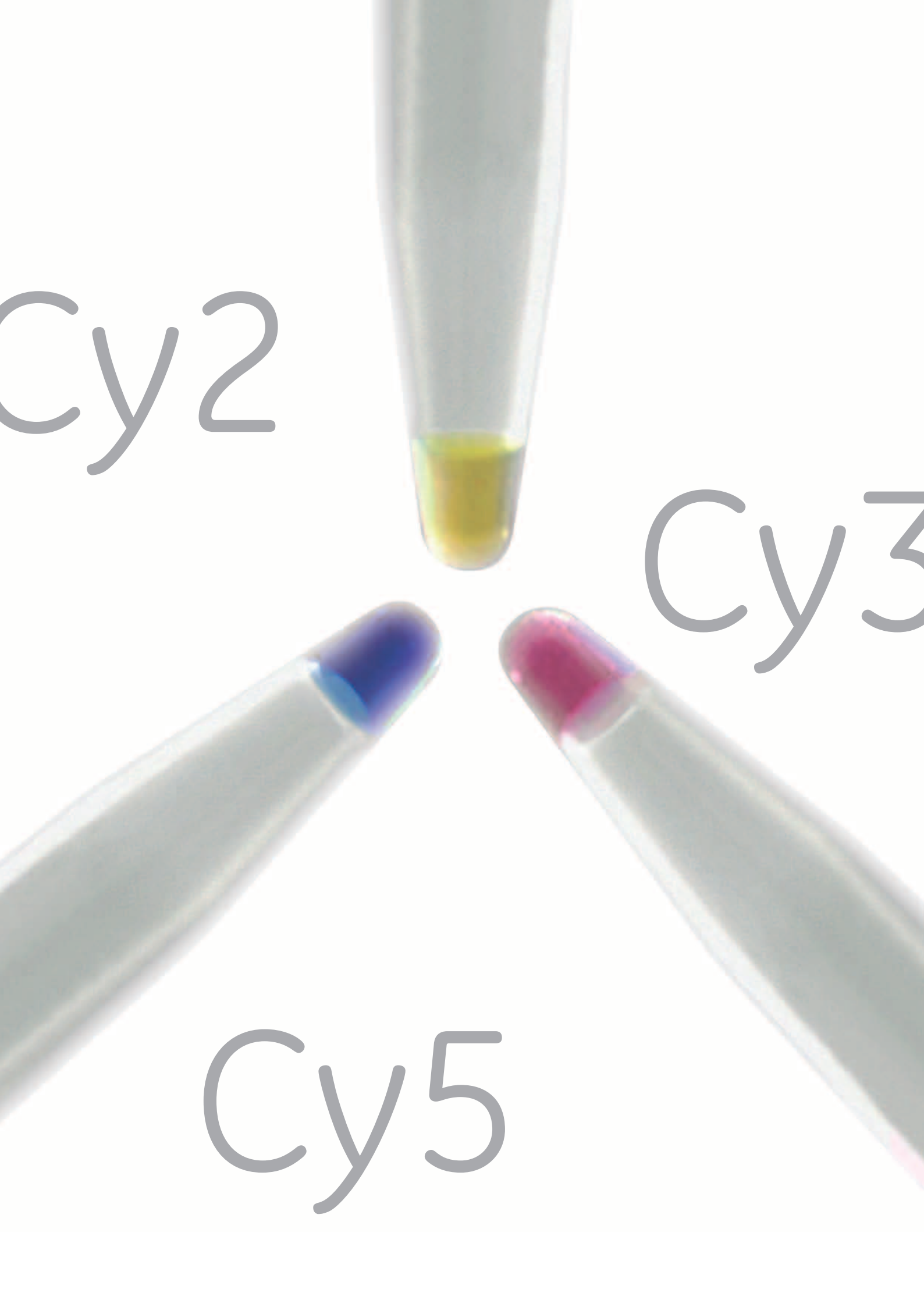
GE Healthcare
Life Sciences

Experience the power of 2-D electrophoresis with 2-D DIGE



imagination at work


Cy2
Cy3
Cy5

Make every spot count

Research is competitive, and you need effective tools to keep up. 2-D DIGE gives you the competitive edge to confidently quantitate significant differences in protein abundance.

Are you looking for protein markers or post-translational modifications in diseased tissues or cell lines? Proteins that are up- or downregulated in response to drug treatment or exposure to certain conditions? Diagnostic markers for disease, or molecular markers that can predict a certain biological state?

Whatever protein you're looking for, 2-D DIGE gives you relevant answers so you can be confident about your conclusions.

The power of 2-D DIGE

2-D electrophoresis is a standard technique for visualizing complex protein mixtures from biological samples. However, there are drawbacks since it is time consuming and data quality varies due to gel-to-gel variation.

2-D DIGE enables you to overcome the limitations of conventional 2-D electrophoresis. The ability to multiplex, and the use of an internal standard, allows you to use your time efficiently and extract the most information from every gel.

- The internal standard minimizes gel-to-gel variation, increases statistical accuracy, and facilitates the analysis of large sets of experiments
- Powerful analysis tools accurately quantitate very small changes in protein abundance
- Multiplexing increases throughput and decreases analysis time

"We do not perform standard 2-D electrophoresis anymore. We only do DIGE because it is quicker, cheaper, and gives us higher quality information."

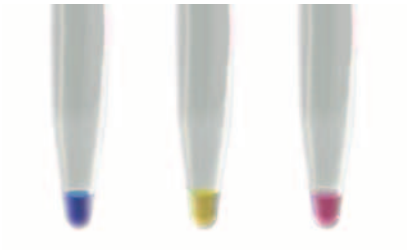
Dr. Richard Burchmore, University of Glasgow

The difference with DIGE

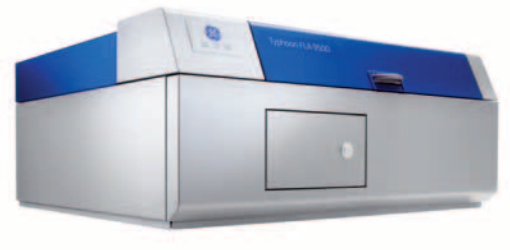
GE Healthcare offers an extensive range of products for both the 2-D electrophoresis and 2-D DIGE workflows. The practical differences between the two workflows are in the protein labeling and image analysis steps.

While proteins are post-stained in conventional 2-D electrophoresis, proteins in 2-D DIGE are labeled before electrophoresis with size- and charged-matched CyDye™ DIGE Fluors.

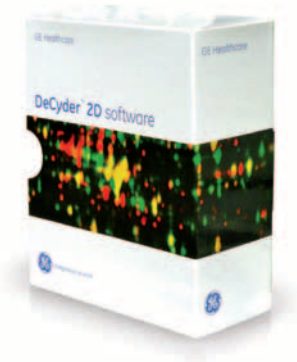
Image analysis of 2-D DIGE gels is performed with a biomolecular imager such as Typhoon™ FLA 9500, that detects multiplex fluorescence. Analysis is performed using DeCyder™ 2-D Differential Analysis Software, optimized for 2-D DIGE.



CyDye DIGE Fluors



Typhoon FLA 9500



DeCyder 2-D Differential Analysis Software

Image analysis of 2-D DIGE is performed with a scanner such as Typhoon FLA 9500 that enables multiplex fluorescence detection.

How does 2-D DIGE work?

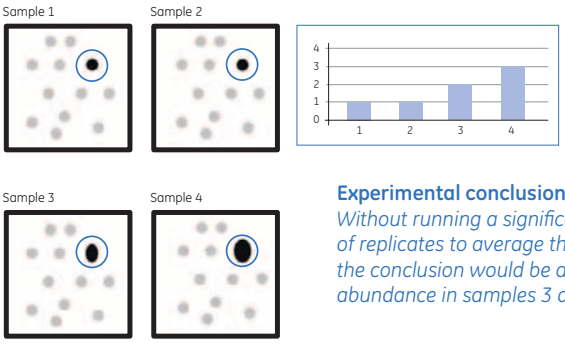
For a technique to be robust, accurate, and quantitative, it is crucial to have a standard for all samples—including those run on different gels—so that they can easily be related to one another. This is a pivotal difference that separates 2-D DIGE from conventional 2-D electrophoresis.

An internal standard, made by pooling aliquots from all the samples in an experiment, is run on every 2-D DIGE gel. This means that there is a standard for every spot on the gel, and that all gels within the same experiment are quantitatively linked.

When running several samples, 2-D electrophoresis can be cumbersome since many technical and biological replicates are required. Because the internal standard virtually eliminates gel-to-gel variation, technical replicates are not necessary when you perform 2-D DIGE—only biological replicates. This means improved statistical reliability with fewer gels, less manual labor, and significant cost savings.

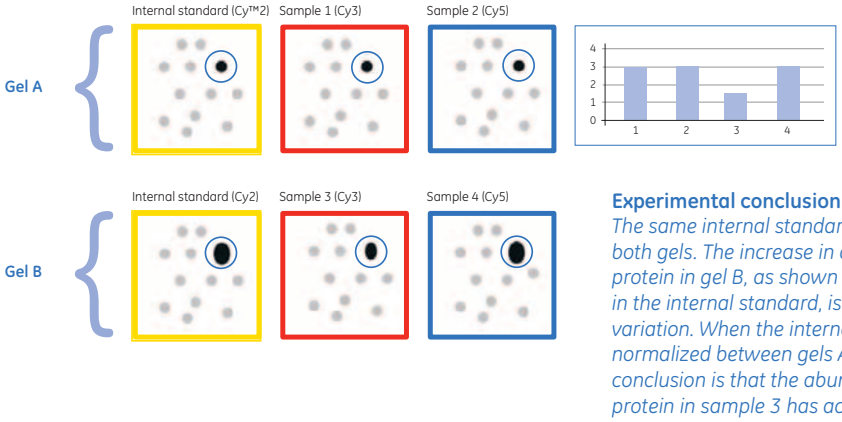
Traditional 2-D electrophoresis
Four different samples run on four different gels

The abundance of this particular protein spot appears to be increasing in samples 3 and 4. Is this increase due to system variation or induced biological change?



Experimental conclusion
Without running a significant number of replicates to average the results, the conclusion would be an increase in abundance in samples 3 and 4.

2-D DIGE using a pooled internal standard
Four different samples, plus one internal standard, on two different gels



Experimental conclusion
The same internal standard is run on both gels. The increase in abundance of protein in gel B, as shown by the increase in the internal standard, is due to gel-to-gel variation. When the internal standard is normalized between gels A and B, the conclusion is that the abundance of protein in sample 3 has actually decreased.

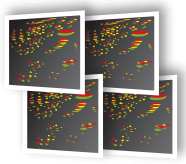
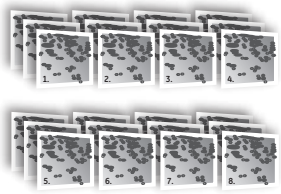
2-D DIGE allows the separation of two samples and an internal standard on every gel. The internal standard is prepared by mixing together equal amounts of each sample in the experiment and including this mixture on each gel.

If you're new to 2-D DIGE, read on to find out about the key differences that make 2-D DIGE a powerful technique.

Get more by doing less

Electrophoresis of multiple samples on the same gel increases efficiency substantially by reducing the number of gels you need to run. What's more, the internal standard improves the quality of your results. For example, a conventional 2-D electrophoresis experiment with 8 samples run in triplicate would require 24 gels. As 2-D DIGE has negligible experimental variation, and requires no technical replicates, only 4 gels are needed for the same experiment. This saves you days of work.

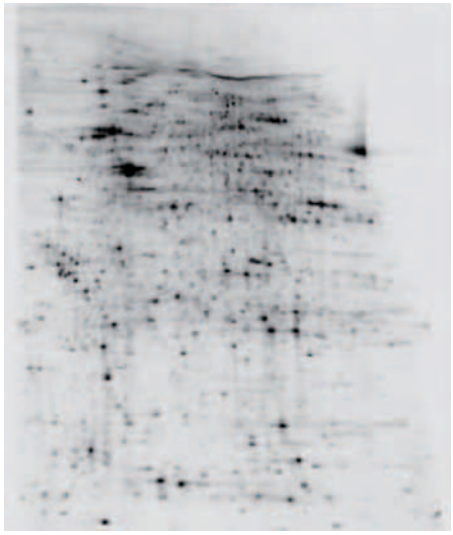
2-D DIGE uses simultaneous detection of multiple, fluorescently labeled samples, or multiplexing. Three CyDye DIGE Fluors are available (Cy2, Cy3, and Cy5), that allow you to run two samples and the internal standard on each gel.

	2-D DIGE	2-D electrophoresis
Experimental variation	Low	High
Quantitative accuracy	High	Low (no internal standard)
Detection limit	CyDye DIGE Fluor ~ 0.25 ng protein	Silver stain ~ 1.0 ng protein
Dynamic range	~ 10^4	~ 10^1
Multiplexing	Yes	No
Time-consuming replicates	Only biological replicates	Large numbers of technical replicates
Number of gels needed for 8 samples	4 gels with 2 samples = 8 no technical replicates needed. 	8 gels × 3 technical replicates = 24 
Running cost per experiment	$	$$$$$

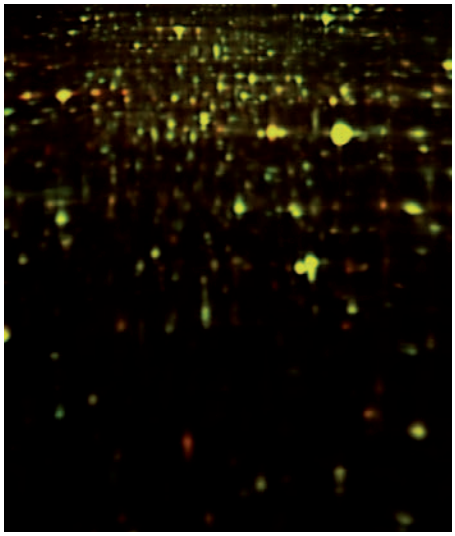
Find what you're looking for

CyDye DIGE Fluors are sensitive, photostable fluorescent dyes for 2-D DIGE. The dyes can detect as little as 0.25 ng of protein which makes them more sensitive than silver staining. They also have a wide dynamic range over approximately four orders of magnitude.

The high sensitivity and wide dynamic range of CyDye DIGE Fluors enables the detection and accurate quantitation of both high and low abundant proteins.



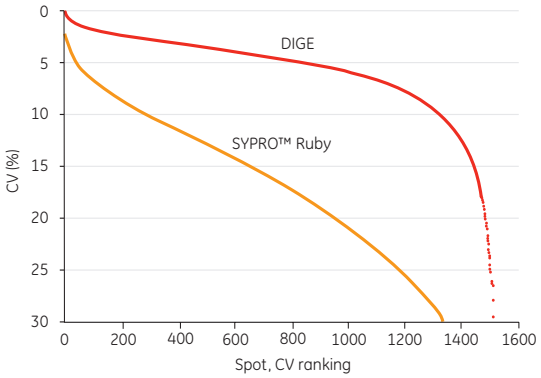
2-D gel separation of a sample pre-labelled with CyDye



2-DIGE overlay image of samples labelled with Cy3 and Cy5.

Results you can defend

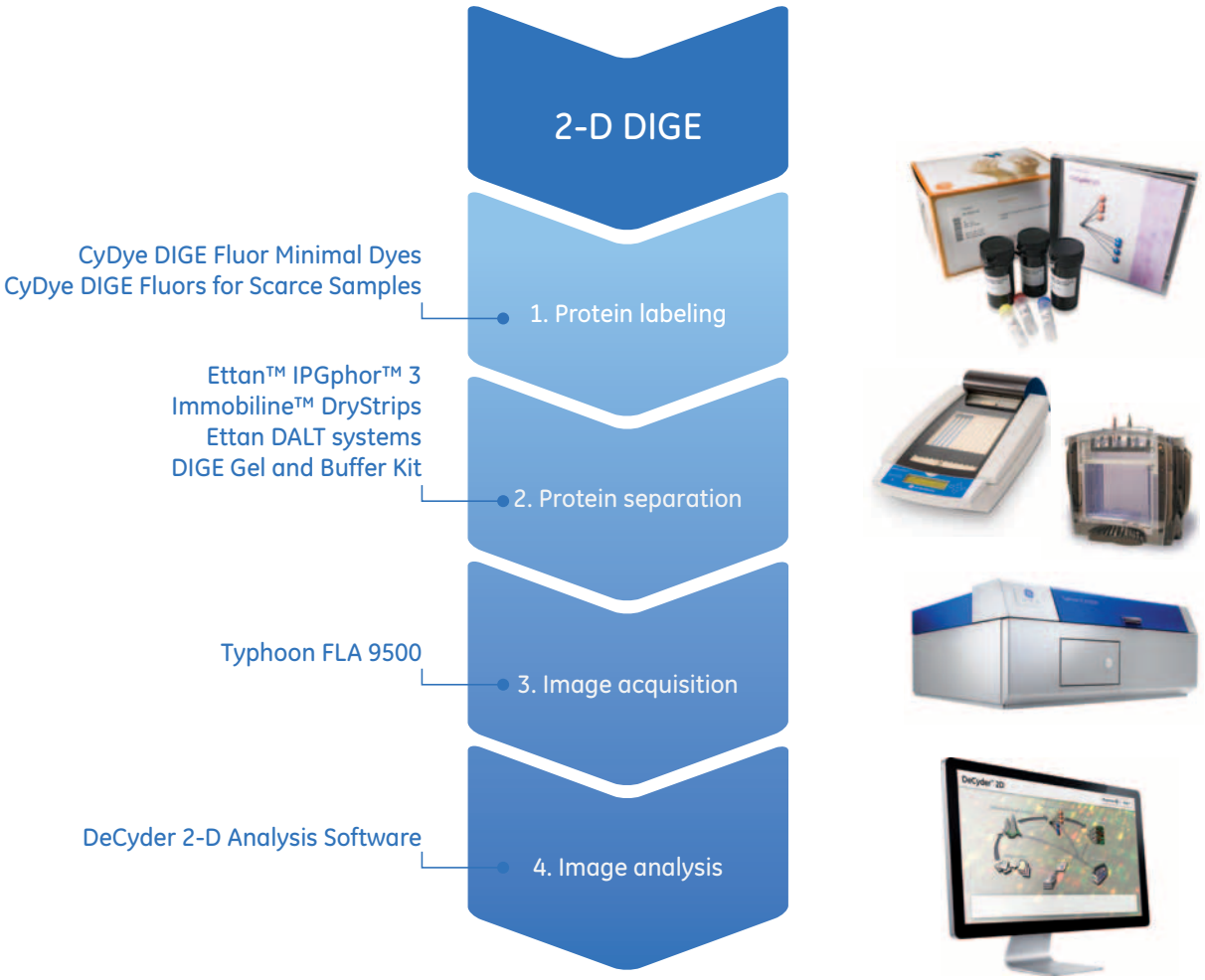
The level of reproducibility and statistical confidence with 2-D DIGE ensure conclusions you can rely on. DeCyder 2-D Differential Analysis Software uses the internal standard to derive data from within gels and between gels in the same experiment. This powerful software package allows you to achieve detection of < 10% differences between samples with > 95% statistical confidence, within minutes.



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.

High throughput tools for 2-D DIGE

For over 30 years, our products for electrophoresis have been trusted by scientists for their high-quality and reproducibility. Our 2-D DIGE products give you high throughput, uncompromised quality, and leave you more time for other important tasks.



Protein labeling

Sensitive and convenient CyDye DIGE Fluors

CyDye DIGE Fluors Cy2, Cy3, and Cy5 are sensitive, spectrally resolvable dyes for 2-D DIGE. They are size-and charge-matched for consistent co-migration of prelabeled samples.

CyDye DIGE Fluor minimal dyes label lysine and are suitable for all sample types. Each labeling reaction requires 50 µg of protein sample. When little sample is available, use CyDye DIGE Fluor Labeling Kit for Scarce Samples.

Protein separation

Robust separation in the first dimension with Immobiline DryStrip gels

Immobiline DryStrip precast gels give reproducible protein separation in the first dimension. Used together with the matching IPG buffers, improved Immobiline DryStrip Gels provide outstanding resolution, reproducibility, and spot definition.

The strips are available, in different lengths, and in linear and nonlinear ranges. IPGbox Kit allows oil-free rehydration of up to 12 IPG strips, independently and simultaneously, for increased efficiency.

Speed and throughput with Ettan IPGphor 3 IEF system

Ettan IPGphor 3 IEF system simplifies the handling of multiple samples and increases the speed and reproducibility of first dimension electrophoresis.

Ettan IPGphor Manifolds reduce hands-on time for running up to 12 IPG strips simultaneously. The ceramic Ettan IPGphor Manifold is specially designed for stringent applications to prevent hot spots that can distort 2-D spot maps, while Ettan IPGphor Manifold Light is made from a robust and lightweight polymer for routine screening applications.

High resolution in the second dimension

In a few hours, Ettan DALT*six* Large Vertical System can run up to six large labcast or precast gels (26 × 20 cm) in the second dimension.

DIGE Gel and Buffer Kit, precast polyacrylamide gels and buffer, give high resolution spot maps and reproducibility. DIGE Gel and DALT electrophoresis systems are compatible with 18 or 24 cm Immobiline DryStrip Gels.

Image acquisition

Cutting edge imaging with Typhoon FLA 9500

Typhoon FLA 9500 is a high performance biomolecular imager optimized for 2-D DIGE multiplex imaging.

The system offers outstanding sensitivity and provides a linear response over five orders of magnitude, enabling precise quantitation, and a 10 µm pixel resolution for high resolution.

Designed for throughput and ease-of-use, Typhoon FLA 9500 has a large scanning area to accomodate two large-format DIGE gels.

In addition to 2-D DIGE, this versatile system performs filmless phosphor imaging, near infrared, and other fluorescent imaging applications.

Image analysis

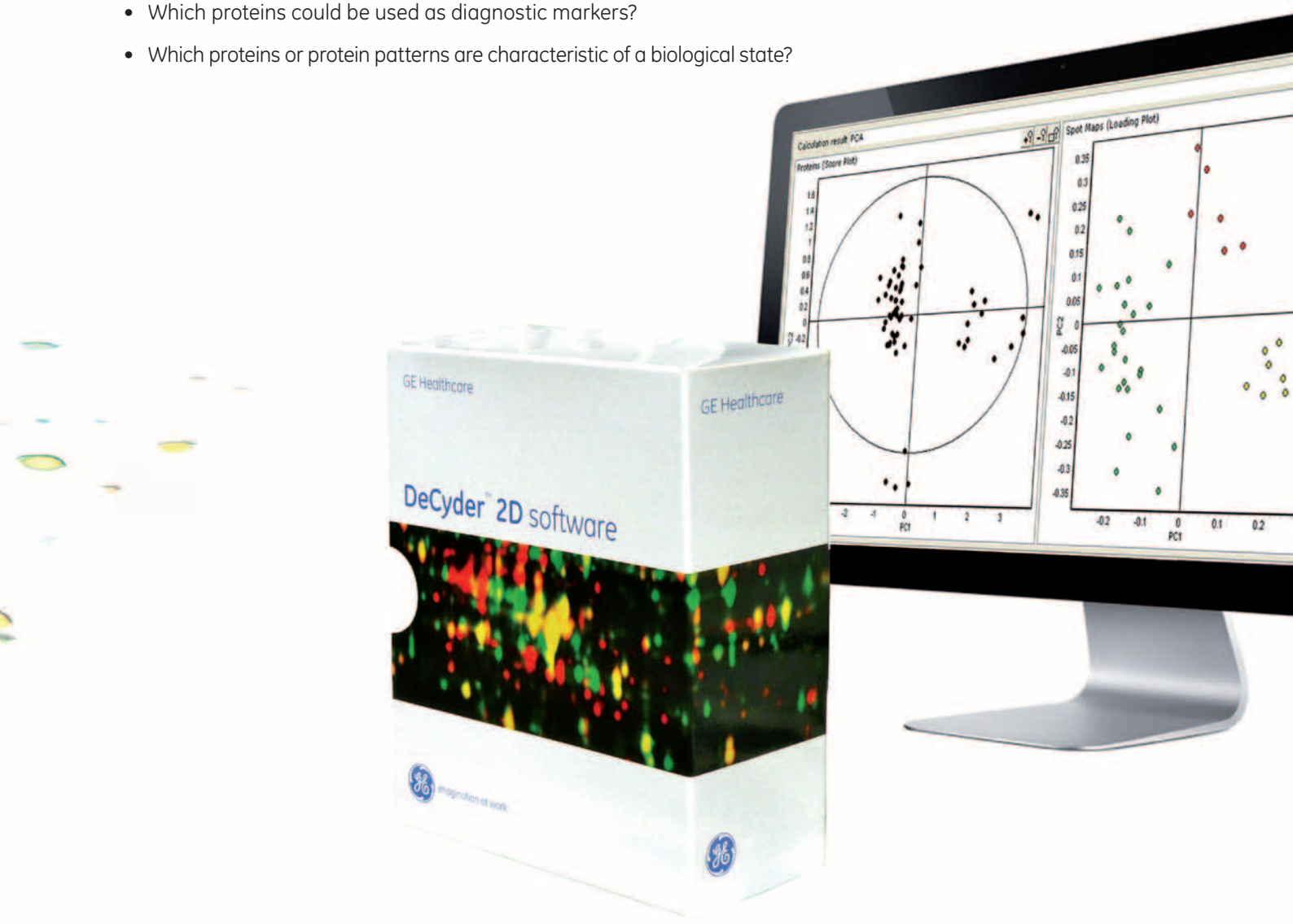
Confidence and ease with DeCyder 2D

DeCyder 2D Differential Analysis Software is optimized to exploit the benefits of internal standards and multiplexed samples.

- Automatically normalize, quantitate, and compare protein spots on a gel or between multiple gels.
- Reduce analysis time from days to minutes, with minimal user-to-user variation.
- Switch quickly between visualization data and results for easy usability.
- Link to databases that put your results in a biological context. Reveal patterns in protein expression data, and gain information about protein function, pathways, and relationships.

Explore key questions such as:

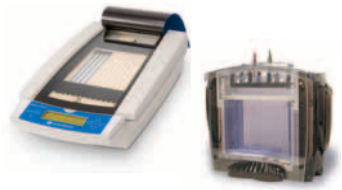
- Which proteins behave similarly or show similar expression patterns?
- How many different subgroups exist in the data set? What are the differences in expression between the groups?
- Which proteins could be used as diagnostic markers?
- Which proteins or protein patterns are characteristic of a biological state?



Integrated solutions from start to finish

From sample preparation through to protein identification and validation, GE Healthcare Life Sciences offers tools to simplify your daily work and save you time. Our products reduce manual labor, and work together seamlessly to produce high-quality results.

Our sample preparation kits are comprised of ready-to-use reagents that give reproducible and consistent results.



Protein identification and validation

1. Sample preparation
   - illustra™ TriplePrep Kit, Vivaspin™, 2D Quant Kit, 2-D Protein Extraction Buffers, 2-D Clean-Up kit
2. Protein labeling
   - CyDye DIGE Fluors
3. Protein separation
   - Ettan IPGphor 3
   - Immobiline DryStrips
   - Ettan DALT systems, DIGE gels
4. Image acquisition
   - Typhoon FLA 9500
5. Image analysis
   - DeCyder 2D software
6. Spot picking
   - Ettan Spot Picker
7. Identification by MS
8. Validation
   - Amersham™ ECL™ Western Blotting Systems

illustra triplePrep Kit

For rapid, simultaneous extraction and isolation of high-quality genomic DNA, total RNA, and total denatured protein from tissues and cells in less than 1 h. This kit is simple to use and delivers high yield and quality.

Vivaspin sample concentrators

Vivaspin offers fast, nondenaturing concentration of biological samples by membrane ultrafiltration with a molecular weight cutoff (MWCO) selected by the user. Up to 30-fold concentration of the sample can be achieved with recovery of the target molecule typically exceeding 95%.

2-D Protein Extraction Buffers

Solubilization buffers help you prepare high-quality protein lysates to give high-resolution spots in 2-D gel analysis. 2-D Extraction Kit allows you to evaluate all six extraction buffers and find the most suitable buffer for your sample.

2-D Quant Kit

2-D Quant Kit is designed for the accurate determination of protein concentration in samples prepared for electrophoresis techniques such as 2-D electrophoresis, SDS-PAGE, or IEF. The 2-D Quant Kit works by quantitatively precipitating proteins while leaving interfering substances behind.

Tools for protein idenfication and validation

To facilitate protein identification, we offer products for preparing preparative gels, spot picking, and spot validation. Our extensive portfolio for validation includes a wide range of antibodies, reagents, and kits.

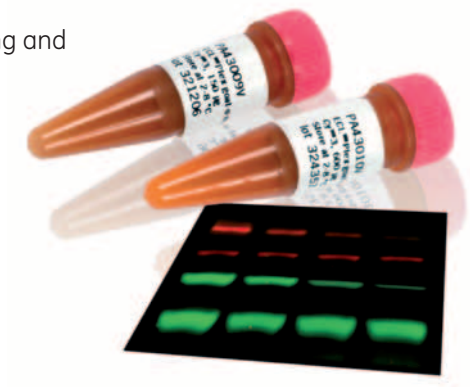
Automatic and robust spot picking

Ettan Spot Picker picks selected protein spots from gels with 99.9% efficiency. Automatically generate a pick list using DeCyder 2D Differential Analysis Software, or pick protein spots using the "click-and-pick" function for built-in flexibility.

Validation with Amersham ECL detection systems

GE Healthcare's Amersham ECL detection systems for Western blotting have delivered speed, sensitivity, and versatility since 1990. A range of detection systems are available, for both chemiluminescent and fluorescent applications.

- High sensitivity detection of low abundant proteins and posttranslational modifications
- Stable signal improves reproducibility
- A broad dynamic range
- Simultaneous detection of more than one protein without stripping and reprobing with ECL Plex



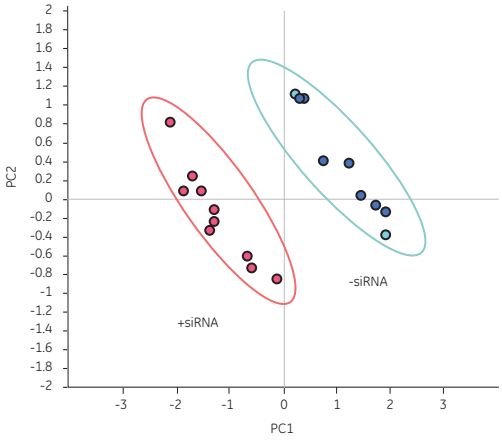
Conclusions you can defend

The quantity of data produced by 2-D electrophoresis can be overwhelming. That's why the combination of an internal standard and optimized analysis software is so powerful. 2-D DIGE gives you clear, biologically relevant results so that you can make the right conclusions.

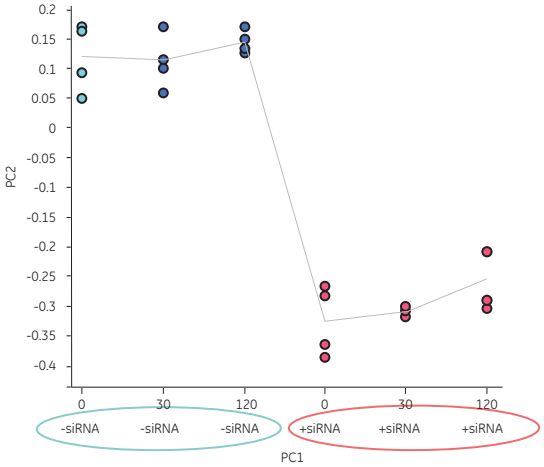
Finding new cancer targets with 2-D DIGE and siRNA

Small interfering RNA (siRNA) are known to interfere with gene expression so that proteins are over- or under-expressed. The prostate cancer cell line (PC-3U), was stimulated with a growth factor and then treat with or without siRNA. 2-D DIGE was used to evaluate if there was differential expression of proteins between the control and siRNA treatment at 0, 30 and 120 minutes. A two-way ANOVA design was used to determine the significance of protein expression and protein interaction under the conditions applied in the study.

The use of 2-D DIGE provided a promising approach to the study of siRNA with an end to improve cancer treatment. So far, 138 differentially regulated proteins have been identified. The experimental analysis revealed a total of 300 proteins that were affected either by the presence of siRNA, the growth factor treatment or by a combination of the factors and time.



The control and siRNA treated PC-3U cells cluster separately in the PCA plot revealing different patterns of protein expression.

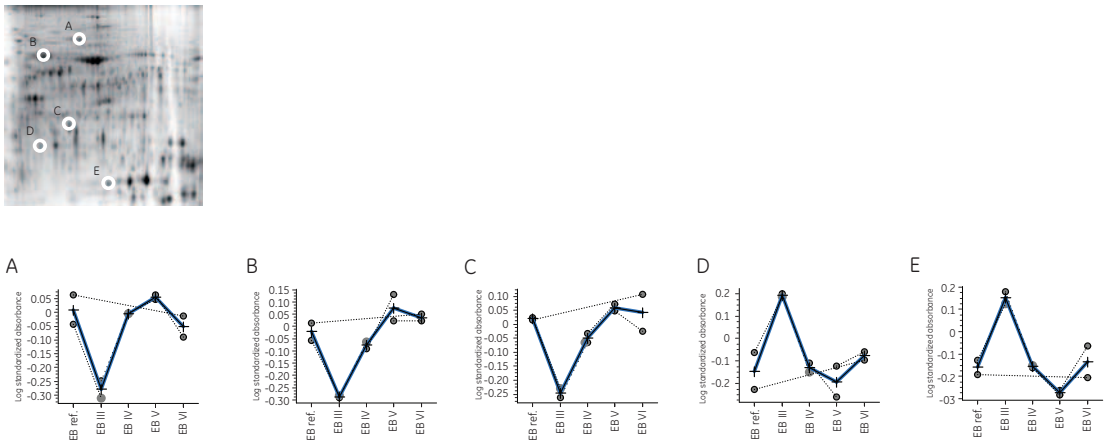


An example of a down-regulated protein spot after siRNA treatment with growth factor stimulation having a minor effect.

Rapid optimization with 2-D Protein Extraction Buffers

Plant tissue is challenging material, due to a high content of interfering components and a low protein content. Finding the right buffer for efficient protein extraction is straightforward with 2-D Protein Extraction Trial Kit. Resuspend the six different extraction buffers included in the kit and extract your samples to see which buffer suits your needs.

The study below shows how extraction of protein from bean leaves was optimized.
A 1-D gel showed that Protein Extraction Buffer-VI (EB-VI) yielded more total protein and a cleaner background. Protein extracts obtained using different extraction buffers were run on 2-D DIGE gels and DeCyder data confirmed that EB-VI gave excellent spot intensities.

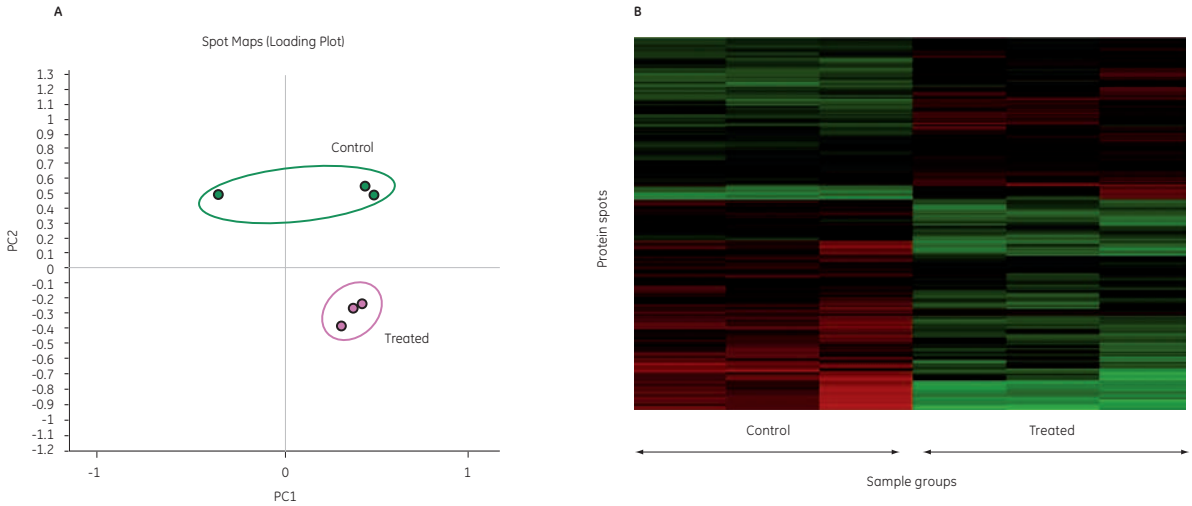


Spot intensities for a subset of spots were analyzed in DeCyder 2D Analysis Software and compared for protein extracts obtained using different 2-D Protein Extraction Buffers. DeCyder 2D results confirmed that 2-D Protein Extraction Buffer-VI gave efficient protein extraction.

Discover new PET tracers to analyze treatment efficacy

The multicellular tumor spheroid (MTS) cell culture model allows cells to grow three-dimensionally, displaying characteristics similar *in vivo* to cell growth. Human glioblastoma cells were cultured in a spheroid model and treated with the cancer drug, doxorubicin.

In this study, 2-D DIGE was used to analyze differences in protein abundance between control and drug-treated cells. Differentially expressed proteins were investigated to identify a protein marker that could be used as a PET tracer for analysis of patient's responses to doxorubicin. Using DeCyder 2D, 213 proteins were differentially expressed, several of which seem to be involved in protein folding and apoptosis.



Analysis with DeCyder 2D software: (A) PCA indicated that the control and treated spheroids clustered separately. (B) Hierarchical clustering analysis of 213 differentially expressed proteins showed that one set of proteins were up-regulated (red) and another set of proteins were down-regulated (green).

Explore the possibilities

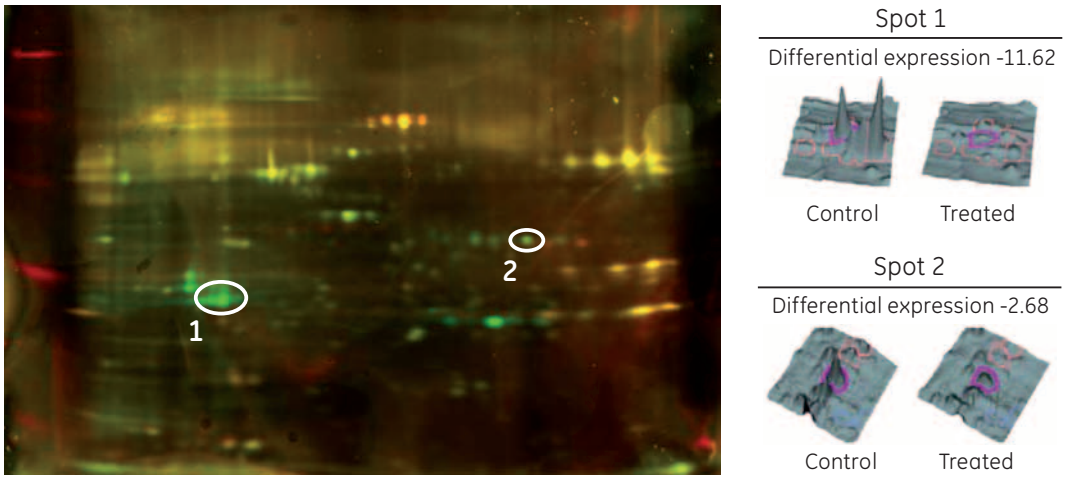
By using 2-D DIGE in combination with enrichment techniques, you can push the limits of detection even further. Maximize the possibilities of detecting small, but significant changes in protein abundance involved in cell signaling, disease mechanisms, response to treatment, and more.

Affinity enrichment for sensitive protein detection

Deregulated tyrosine kinase activity leads to chronic myeloid leukemia (CML). However, tyrosine phosphorylation accounts for only a few percent of all phosphorylation, which means that tyrosine phosphorylated (pTyr) proteins have low abundance in cells. To detect pTyr proteins from a CML cell line, they were enriched using Protein G Mag Sepharose™ magnetic beads.

Increased tyrosine kinase activity in CML is inhibited by the drug Imatinib. In this study, enriched pTyr proteins from control and Imatinib-treated samples were analyzed by 2-D DIGE to identify small differences in pTyr protein abundance.

In total, approximately 150 protein spots were separated from the enriched samples. Of these, ten proteins were downregulated more than two-fold after treatment with Imatinib. Differentially regulated protein spots were picked and identified by mass spectrometry. The results were also confirmed by 2-D Western blotting using ECL Plex.



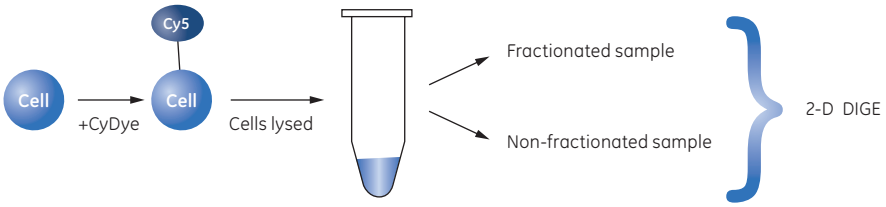
2-D DIGE gel overlay. Green spots demonstrate proteins that were downregulated after treatment. Visualization data from DeCyder 2D compares spot intensities between control and treated samples.

Fast and selective labeling of cell surface proteins

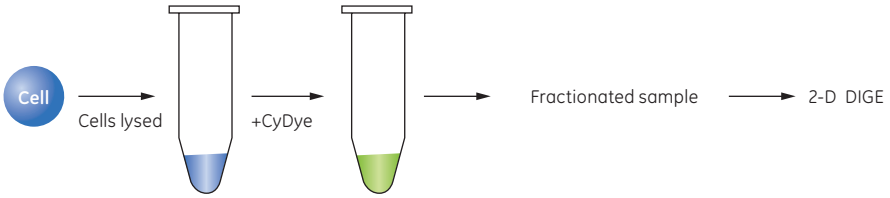
Cell surface proteins are important in cell signaling, and are therefore interesting as potential drug targets. These low abundant proteins constitute 1-2% of cellular proteins which make them difficult to detect without fractionation or enrichment.

Using 2-D DIGE, cell surface proteins can be labeled directly by mixing unlysed cells with CyDye DIGE Fluors. The protocol is rapid and specific, and only fluorescently labels cell surface proteins.

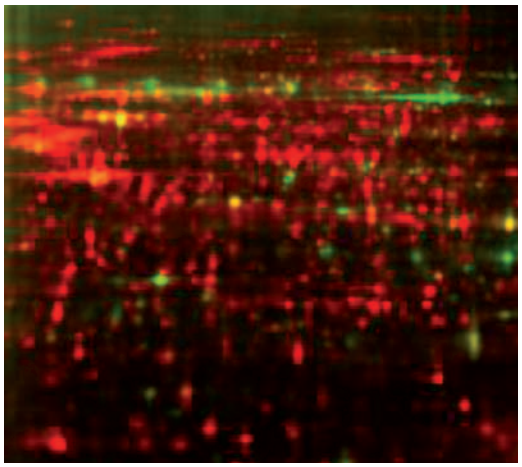
Cell-surface protein labeling protocol



Standard Ettan DIGE protocol



Overview of labeling workflow protocols.



2-D DIGE gel overlay of a Cy5-labeled sample using the cell-surface labeling protocol (red spots) and a membrane fractionated Cy3-labeled sample (green spots) using the standard DIGE labeling protocol followed by membrane fractionation. Only a few membrane proteins are labeled using the standard DIGE labeling protocol (green spots).

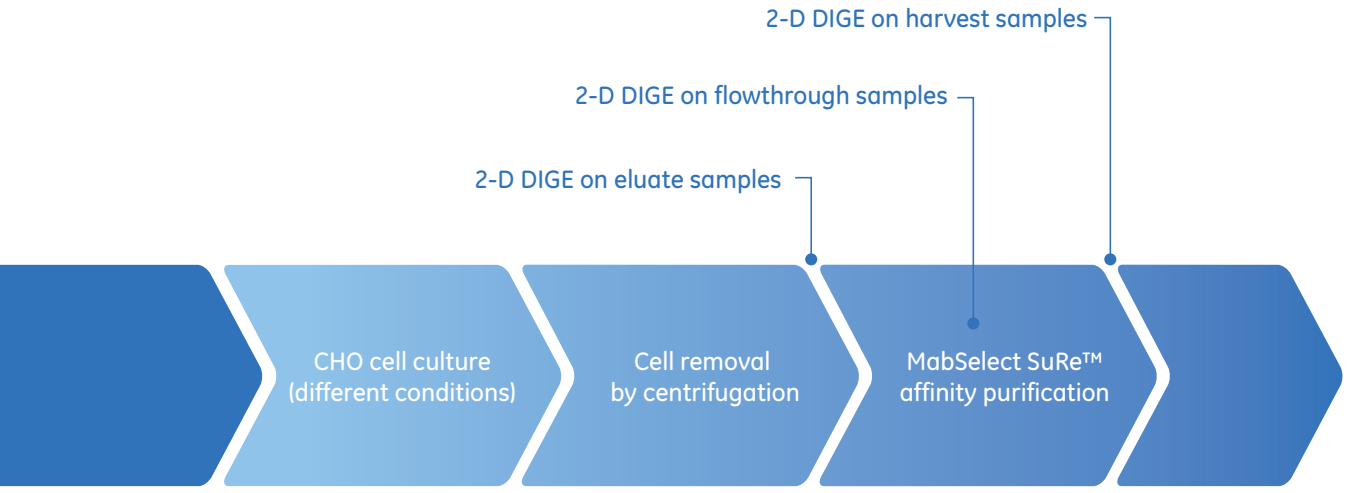
Understanding processes

As regulatory demands get tougher, manufacturers of biopharmaceuticals are required to better understand how their process conditions affect product quality. 2-D DIGE can help by identifying impurities, facilitating process optimization and by providing valuable information for better decision making with Quality by Design.

- Facilitate selection of cell lines, cell clones, or growth media based on downstream effects
- Correlate upstream processes to critical impurities or yield of target protein
- Compare target protein before and after a process change
- Analyze batch-to-batch variation

Investigate HCP patterns for tighter process control

Host cell protein (HCP) contamination was investigated for a monoclonal antibody (MAb) purification. CHO cells expressing the MAb were cultured under different conditions and the HCP pattern of the harvest samples, flowthrough samples, and purified MAbs were analyzed using 2-D DIGE. By quantitatively linking upstream and downstream processes, a better understanding of the production process was attained.

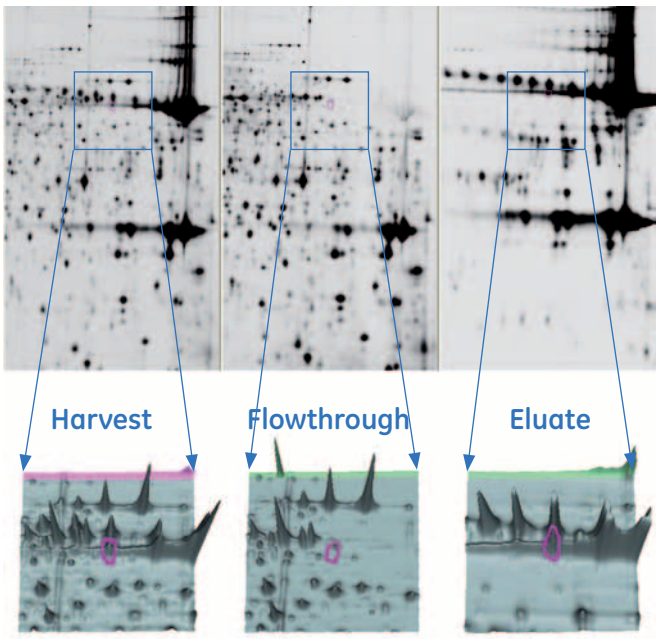


How upstream processes affect downstream results

Since protein spot maps from upstream and downstream samples will differ, three reference proteins were introduced to normalize protein abundance levels between samples.

As expected, most HCP was observed in the flowthrough and a small amount co-eluted with the MAb. The DeCyder software revealed that purified MAb samples from the different groups of culture conditions were clustered and showed slightly different HCP patterns.

Significant differences in protein abundance were found for 96 spots. Thirty-six of these spots were MAb-related, leaving 60 possible HCPs. These proteins were then be analyzed in DeCyder 2D to correlate cell culture conditions with HCP profiles and identify HCP that were difficult to remove during MAb purification.



Top: 2-D DIGE spot maps of the harvest, flowthrough, and eluate fractions from a MAb purification.
Bottom: Spot intensities of a section of the 2-D maps are viewed in DeCyder 2D to visualize how spot patterns differ between upstream and downstream samples.

Ordering information

Product	Quantity	Code no.
Sample preparation kits and reagents		
2-D Protein Extraction Buffer Trial Kit	1 × for 50 ml	28-9435-22
2-D Protein Extraction Buffer-I	1 × for 50 ml	28-9435-23
2-D Protein Extraction Buffer-II	1 × for 50 ml	28-9435-24
2-D Protein Extraction Buffer-III	1 × for 50 ml	28-9435-25
2-D Protein Extraction Buffer-IV	1 × for 50 ml	28-9435-26
2-D Protein Extraction Buffer-V	1 × for 50 ml	28-9435-27
2-D Protein Extraction Buffer-VI	1 × for 50 ml	28-9435-28
illustra TriplePrep Kit	50 preps	28-9425-44
2-D Quant Kit	500 assays	80-6483-56
2-D Clean-Up Kit	50 samples	80-6484-51
Sample labeling		
CyDye DIGE Fluor minimal dye labeling kit	2 nmol	28-9345-30
CyDye DIGE Fluor, minimal labeling kit	5 nmol	25-8010-65
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
Sample separation, first dimension		
DeStreak Rehydration Solution	5 × 3 ml	17-6003-19
DeStreak Reagent	1 ml	17-6003-18
Ettan IPGphor 3 Isoelectric Focusing Unit	1	11-0033-64
Immobiline DryStrip Kit	1	18-1004-30
IPGbox	1 IPGbox + IPGbox Kit	28-9334-65
IPGbox Kit	10 Reswell Trays + IPGbox Insert	28-9334-92
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
Ettan IPGphor Manifold, Complete	1	80-6498-38
Ettan IPGphor Manifold, Light Complete	1	11-0026-88
Multiphor™ II Electrophoresis System	1	18-1018-06

Product	Quantity	Code no.
Sample separation, second dimension		
Ettan DALT*six* Electrophoresis Unit, 115V	1	80-6485-08
Ettan DALT*six* Electrophoresis Unit, 220V	1	80-6485-27
EPS 601 Power Supply 18-1130-02	1	18-1130-02
DALT*six* Gel Caster	1	80-6485-46
DIGE Gel	3 pk	28-9374-51
DIGE Buffer Kit	2 × DALT6 runs	28-9374-52
Low-fluorescence Glass Plates, 27 × 21 cm (including spacers)		80-6475-58
MultiTemp™ IV, Thermostatic Circulator, 115 V	1	28-9941-72
MultiTemp IV, Thermostatic Circulator, 230 V	1	28-9941-71
Image acquisition		
Typhoon FLA 9500		29-0040-80
Image analysis		
DeCyder 2D 7.2 Preinstalled computer 1-User license		28-9856-57
DeCyder 2D 7.2 Concurrent network license		28-9854-18
DeCyder 2D 7.2 SPN 1 Concurrent network license		28-9854-11
DeCyder 2D 7.2 SPN 1 Concurrent network license Upgrade		28-9854-17
DeCyder 2D Oracle 11g 5 user licenses		28-9435-88
2-D Electrophoresis: Principles and Methods handbook		80-6429-60
Ettan DIGE System User Manual		18-1173-17

Immobiline DryStrip Gels for IEF†

pH range	Quantity	18 cm	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

† *IPG strips are also available in 7, 11 and 13 cm strip lengths. For information, contact your local GE Healthcare representative or consult www.gelifesciences.com.*

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