Nothing matches Ettan DIGE for accuracy

18-1176-07 AA

Amersham
Biosciences

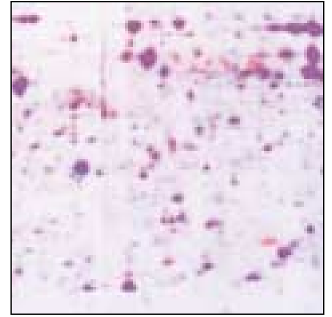
The differences you see
in protein abundance are

real

Ettan DIGE: raising the standard of 2-D

Accurate results lead to confident conclusions and the right decision

Ettan™ DIGE system uses 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) —the new benchmark for protein abundance analysis. Many of the world's leading pharmaceutical and academic centers-of-excellence have switched to Ettan DIGE system because of the unrivaled accuracy it provides.



The reasons for this shift are quite simple; Ettan DIGE system brings unique levels of statistical confidence and reliability to 2-D electrophoresis results. Each protein spot has its own internal standard, which provides unparalleled accuracy and ensures that the differences you see in protein abundance are real.

Standardize your 2-D results

Ettan DIGE system uses multiplexing, the simultaneous co-separation of multiple, fluorescently labelled samples including an internal standard on a single gel. This is the only effective way to remove gel-to-gel variation, thereby significantly increasing accuracy and reproducibility.

Cost and time are relative, but accuracy is absolute

The level of reproducibility and statistical confidence generated by Ettan DIGE system creates another distinct advantage—results you can rely on from far fewer gels. Ettan DIGE system offers savings in consumables and time because only a fraction of the gels normally used to measure protein abundance differences in 2-D electrophoresis are required to achieve dependable results.

Ettan DIGE system provides these important benefits

- **Standardization** – An internal standard is used to deliver the lowest possible gel-to-gel variation
- **Accuracy** – Ensures that the smallest possible real differences in protein abundance are detected with unparalleled statistical confidence
- **Reproducibility** – Excellent quantitative reproducibility leads to consistent and comparable conclusions
- **Efficiency** – Delivers increased throughput and significantly reduces analysis time
- **Proven method** – 2-D DIGE has rapidly become well established in leading laboratories worldwide

Results

you can trust

Ettan DIGE is the only system with an internal standard for every spot on every gel.

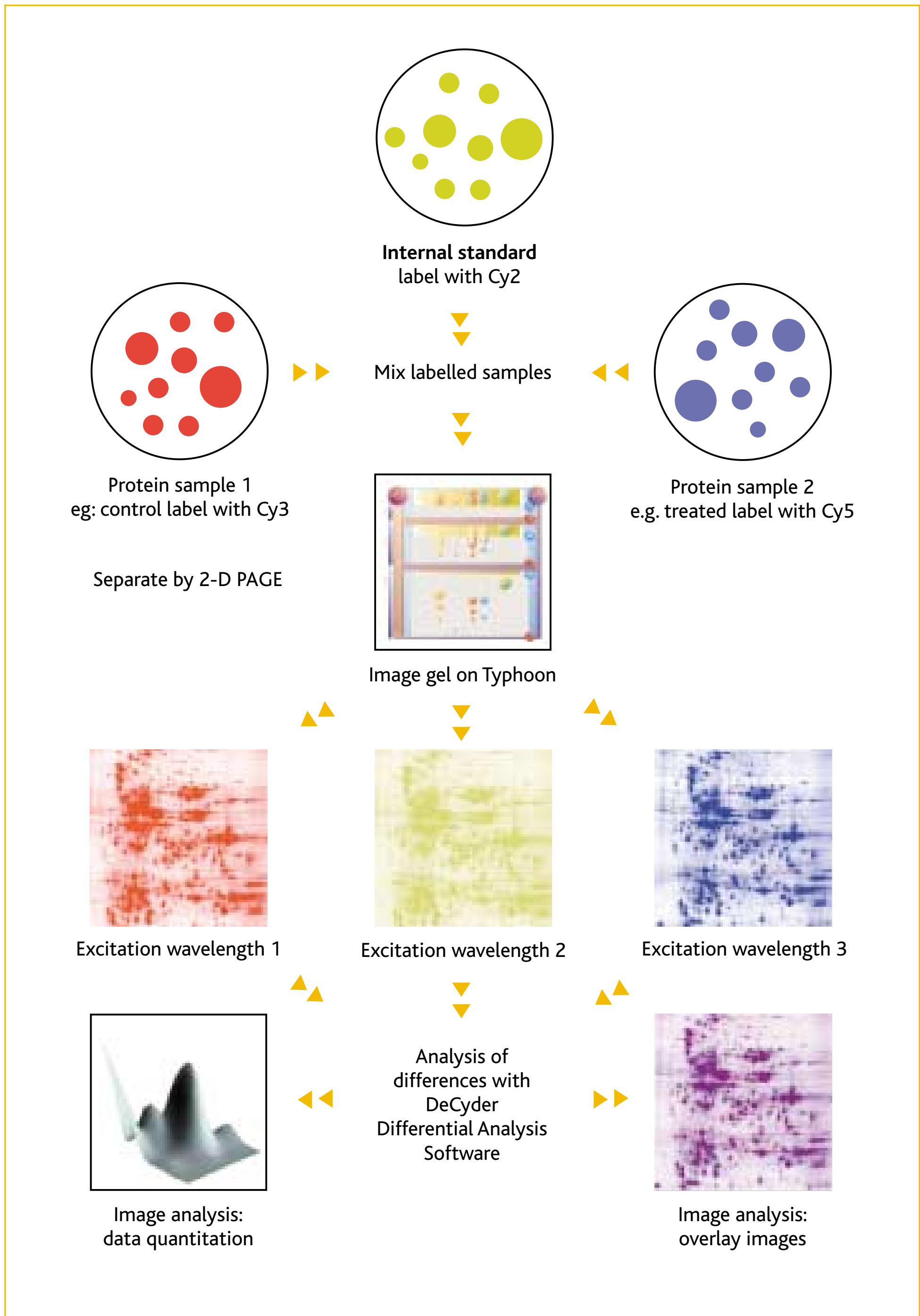
Ettan DIGE system uses prelabelling of protein samples with size- and charge-matched, spectrally resolvable CyDye™ DIGE fluors. Add DeCyder Differential Analysis Software, our novel co-detection software, and unique experimental design, and you get certainty in 2-D electrophoresis. The internal standard is labelled with one of the CyDye DIGE fluors (e.g. Cy™2) while the experimental and control samples are labelled with Cy3 and Cy5 CyDye DIGE fluors respectively. The internal standard and both samples are subsequently combined and run in the same first- and second-dimension gel.

After separation on Ettan IPGphor™ II or Multiphor™ II in the first dimension and Ettan DALT*twelve* or Ettan DALT*six* electrophoresis systems in the second dimension, samples are scanned with Typhoon™ 9400 Variable Mode Imager.

Differences in protein abundance are then accurately quantitated using DeCyder™ Differential Analysis Software. DeCyder software uses the internal standard to derive data from within gels and then between gels, eliminating gel-to-gel variation. Within minutes, DeCyder software allows you to achieve routine detection of <10% differences between samples with >95% statistical confidence.

The ability to multiplex prelabelled samples AND eliminate variation within and between gels makes Ettan DIGE system unique. Efficient analysis with intuitive software and the internal standard is the key to the system's extraordinary accuracy when comparing protein abundance levels.

With Ettan DIGE system, your goals are more readily achieved with results you can trust.



Internal standard
label with Cy2
Mix labelled samples
Protein sample 1
eg: control label with Cy3
Protein sample 2
e.g. treated label with Cy5
Separate by 2-D PAGE
Image gel on Typhoon
Excitation wavelength 1
Excitation wavelength 2
Excitation wavelength 3
Analysis of
differences with
DeCyder
Differential Analysis
Software
Image analysis:
data quantitation
Image analysis:
overlay images

Conclusions

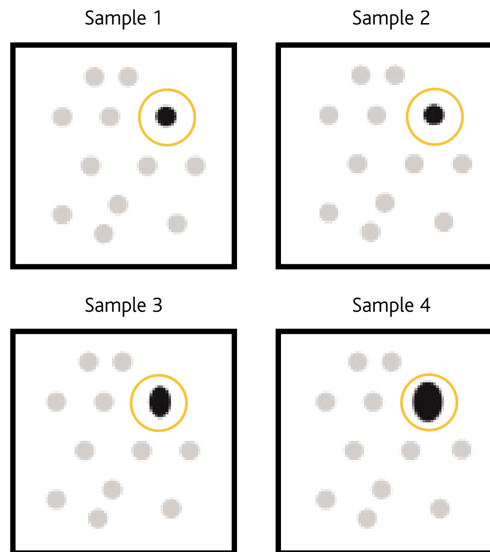
you can defend

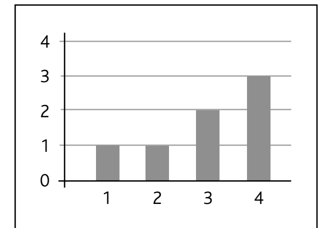
The use of the internal standard leads to accurate conclusions.

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?



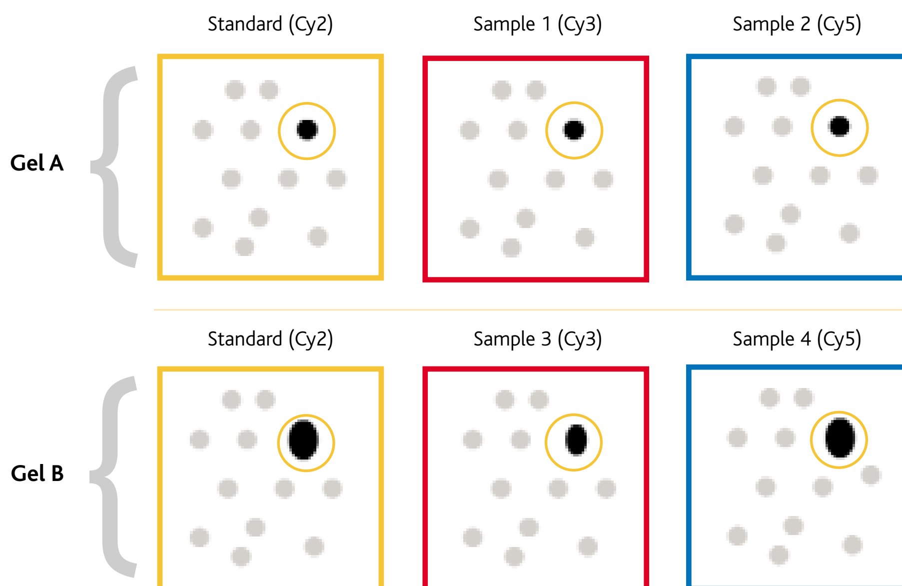


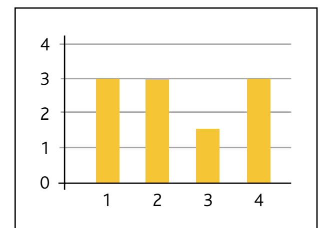
Experimental Conclusion

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

Ettan DIGE using an 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 the abundance of protein in sample 3 has actually decreased.

A complete system

for protein difference analysis

Sample labelling

CyDye DIGE fluors – Designed specifically for Ettan DIGE system, these dyes are size- and charge-matched, as well as spectrally distinct, offering bright and intense colors with narrow excitation and emission bands. This allows co-separation of different CyDye DIGE fluor-labelled samples in the same gel and ensures that all samples will be subject to exactly the same first- and second-dimension electrophoresis running conditions, limiting experimental variation and ensuring accurate within-gel matching.

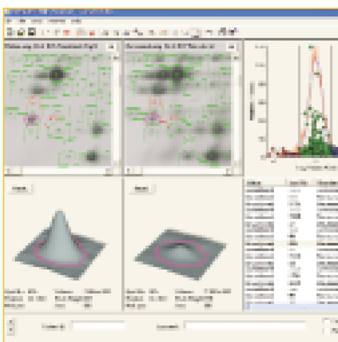
2-D electrophoresis

Ettan IPGphor II, Multiphor II, and Ettan DALT electrophoresis systems – Simultaneously separate up to three samples on a single 2-D gel with Ettan IPGphor II or Multiphor II in the first dimension and Ettan DALT*twelve* or Ettan DALT*six* in the second dimension.

Image acquisition

Typhoon 9400 Variable Mode Imager – Optimized to image CyDye DIGE fluor-labelled proteins in 2-D DIGE analysis using Ettan DIGE system. Automated multicolor scanning enables detection of multiple samples in the same experiment to ensure accurate analysis and increased throughput. Typhoon 9400 combines the ability to detect an extensive variety of fluors with proven storage phosphor autoradiography technology and direct imaging of chemiluminescence.

Image analysis

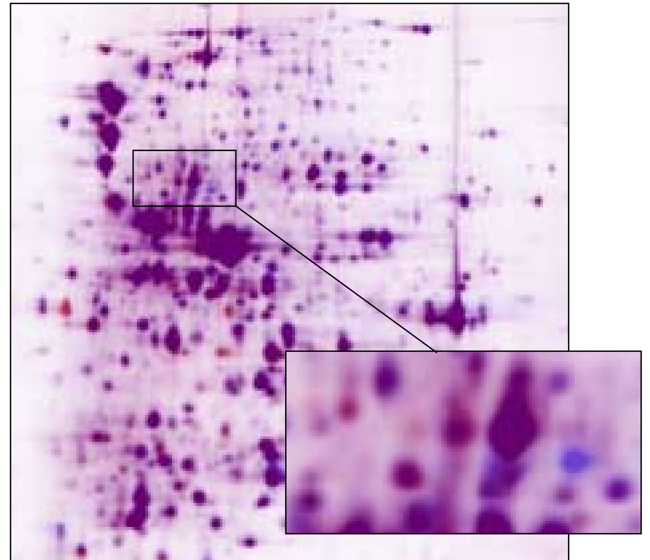


DeCyder Differential Analysis Software – Specifically developed to exploit the benefits of prelabelled, multiplexed samples and the internal standard. The software automatically locates and analyzes multiple samples in a gel and then enables comparative analysis of multiple gels, producing accurate measurement of differential changes in protein abundance. DeCyder software brings statistical confidence and minimal user-to-user variation reducing hands-on analysis time to minutes.

Real
results

2-D DIGE analysis of *E. coli*

Experimental conditions: Control *E. coli* versus *E. coli* treated with 20 mM Benzoic Acid, pH 8. Cell lysates were labelled and separated by 2-D DIGE, images were analyzed using DeCyder Differential Analysis Software. Examples of abundance changes in protein levels identified after MALDI-ToF analysis are indicated.



Combined images. Spots showing red only are proteins induced during treatment (increased abundance). Blue spots represent down-regulated proteins (decreased abundance).

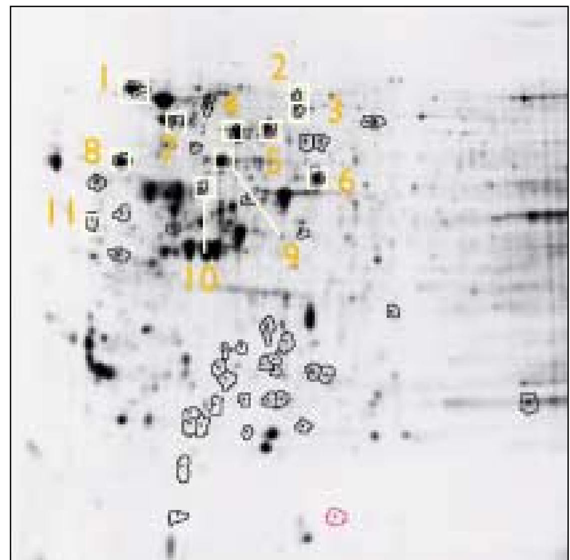
Spots represented as red or blue are obvious differences.

Blue = Cy3 = Control

Red = Cy5 = Benzoic acid treated

2-D DIGE analysis of cancer cells

Transformed and nontransformed human large intestine cell lines were subjected to 2-D DIGE and analyzed using DeCyder Differential Analysis Software. Examples of differentially expressed proteins identified after MALDI-ToF analysis are indicated.



Examples of protein abundance changes.

Theoretical pI/M^r	Average ratio Treated/control	t-test	ID
5.8/55222	-1.19	3.6×10^{-5}	ATP synthase alpha chain
4.9/50194	-1.16	1.0×10^{-5}	ATP synthase beta chain isoform
5.77/50729	-1.11	0.00041	Pyruvate kinase I
5.24/93498	1.16	4.2×10^{-6}	Aconitate hydratase 2 isoform
5.19/34758	-1.29	0.00024	6-Phosphofructokinase isoenzyme 1
5.46/99537	1.16	0.00024	Pyruvate dehydrogenase
4.78/63562	-1.32	9.8×10^{-8}	PEP protein phosphotransferase
5.15/45747	1.12	0.0016	Isocitrate dehydrogenase
5.25/32456	1.18	0.0023	6-Phosphofructokinase isoenzyme 2
5.55/23586	1.22	1.0×10^{-5}	Adenylate kinase
4.83/68984	1.36	8.3×10^{-7}	DNAK protein (HSP70) isoform
4.85/57138	1.12	3.7×10^{-5}	60 kDa Chaperonin CPN60, GROEL isoform

Spot number	Average ratio Treated/control*	t-test	ID
1	1.15	1.5×10^{-6}	ENPL_HUMAN: Endoplasmin precursor
2	-1.11	0.0024	HSGLUCOII: Glucosidase II
3	-1.05	0.016	KU86_HUMAN: ATP-dependent DNA Helicase II 86 kDa subunit
4	1.07	0.00054	HS7C_HUMAN: Heat shock cognate 71 kDa protein
5	1.08	0.00011	GR75_HUMAN: Mitochondrial stress-70 protein precursor
6	1.07	0.00051	ER60_HUMAN: Protein disulfide isomerase ER-60 precursor
7	1.08	0.00040	ER72_HUMAN: Protein disulfide isomerase-related protein precursor
8	1.09	0.00062	PDI_HUMAN: Protein disulfide isomerase precursor
9	1.10	3.5×10^{-5}	P60_HUMAN: Mitochondrial matrix protein P1 precursor
10	-1.10	1.8×10^{-5}	I77403: Tubulin alpha-1 chain human
11	-1.28	0.015	HUMHSP89KD: Heat shock protein 90

* These figures represent increases (positive) or decreases (negative) in abundance e.g. Spot 1 represents an increase in abundance of 15%, with a probability of >99.999% that this is a real difference in abundance.

For more information about Ettan DIGE, visit:

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