

Synchronized solutions for better Western blotting



Welcome to the world of modern protein analysis

Expertise in protein science, today and tomorrow

Over the decades, our scientists and engineers have provided protein researchers with essential technologies. Their contribution to protein science includes several significant developments in the field of protein separation including chromatography and electrophoresis-based methods for protein analysis. Milestones include the world's first electrophoresis system in 1937, the first Biacore™ system for protein interaction studies in 1989, and the first chemiluminescent reagents for Western blotting in 1990. Our detection systems for Western blotting are still the most widely used.

GE Healthcare continues to build on this heritage. Years of hard-won experience have taught us that the only way to detect and quantitate proteins well is to make sure that every step of the process, from the initial sample preparation to the final analysis, is handled optimally.

A range of solutions for Western blotting

The application examples presented here, from the confirmation of the presence of target proteins in chromatography fractions to quantitative, multiplex Western blotting, show strategies that are best suited to each specific challenge. Our attention to manufacturing and rigorous quality control ensure that your high standards for protein detection are consistently met in order to generate reproducible and dependable data.

Whatever your requirements, from routine protein detection to advanced triplex detection or 2-D Western blotting, GE Healthcare has solutions for the entire process, all optimized to work together.

Routine protein detection & analysis

1990 Amersham™ ECL™ makes nonradioactive protein detection possible for the first time

Versatile, quantitative protein measurement

1997 Amersham ECL Plus introduces flexible chemiluminescent and chemifluorescent detection for Western blotting

Efficient use of precious primary antibodies

2002 Amersham ECL Advance™ dramatically decreases primary antibody consumption, enabling efficient use of expensive or scarce reagents

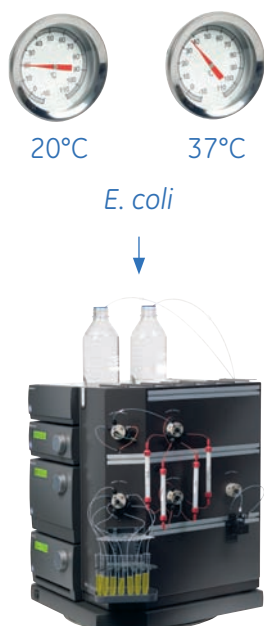
Multiplex protein analysis

2005 Amersham ECL Plex™ brings multiplex, quantitative fluorescent analysis to 1-D and 2-D gels, enabling faster detection and better resolution of protein targets with identical molecular weights

Routine protein detection & analysis

Processes for the production of recombinant proteins in bacteria must deliver high purity and yield. In evaluating methods, it is more important to compare yields or to confirm the presence of the protein of interest in fractions after chromatography runs, than to know precise quantities. Amersham ECL is an excellent choice for detection and analysis of overexpressed and tagged recombinant proteins or other applications where the protein of interest is not limited. It is easy to use and excellent results are achievable with minimal optimization.

- *Reliable results over time from consistent batch-to-batch performance*
- *Visual results using Amersham Hyperfilm™ ECL*
- *Imaging solution for QC/QA labs with data audit, data access control and installation and operational qualification (IQOQ) procedures*
- *Minimal optimization based on validated protocols*



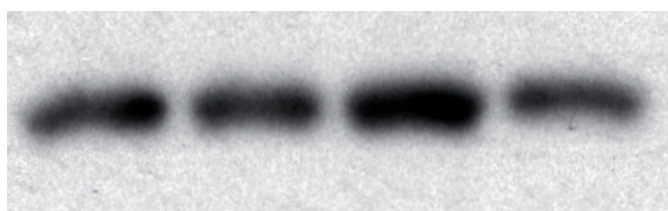
How to optimize your purification strategy before scale-up

When scaling up to produce larger quantities of protein, the production process is developed on a small scale to optimize for high purity and yield. Typically, an optimization experiment might investigate how different induction temperatures affect the yield of recombinant histidine-tagged green fluorescent protein (GFP) produced in *E. coli*. Following purification, electrophoresis and blotting, the eluted samples were analyzed using Amersham ECL.

Detection using Amersham ECL provides a reliable means to compare concentrations of the target protein under a range of conditions. Batch-to-batch performance is highly consistent, adding to the reliability of the final result.

HisTrap™ HP

HiTrap™ Capto™ Q



20°C

37°C

20°C

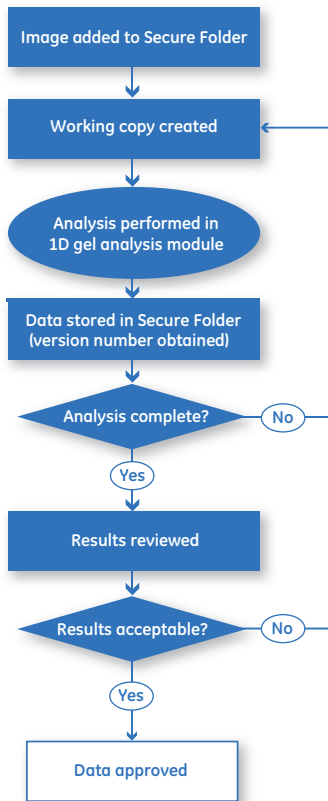
37°C

Detection of recombinant His-GFP with Amersham ECL and Amersham Hyperfilm ECL, for visual comparison of protein yields after different induction temperatures (20°C or 37°C) and purification media (HisTrap HP (1ml) or HiTrap Capto Q (5 ml) on ÄKTAexplorer™ 100). The highest yield of His-GFP was achieved using an induction temperature of 20°C and purification on HiTrap Capto Q.

Following electrophoresis and blotting, the eluted samples were quantitated using Amersham ECL. The highest concentration of His-GFP was achieved using an induction temperature of 20°C and purification on HiTrap Capto Q. In this case, a simple visual analysis of alternative purification strategies to help select the optimal method before scale-up gives sufficient information for the task at hand.

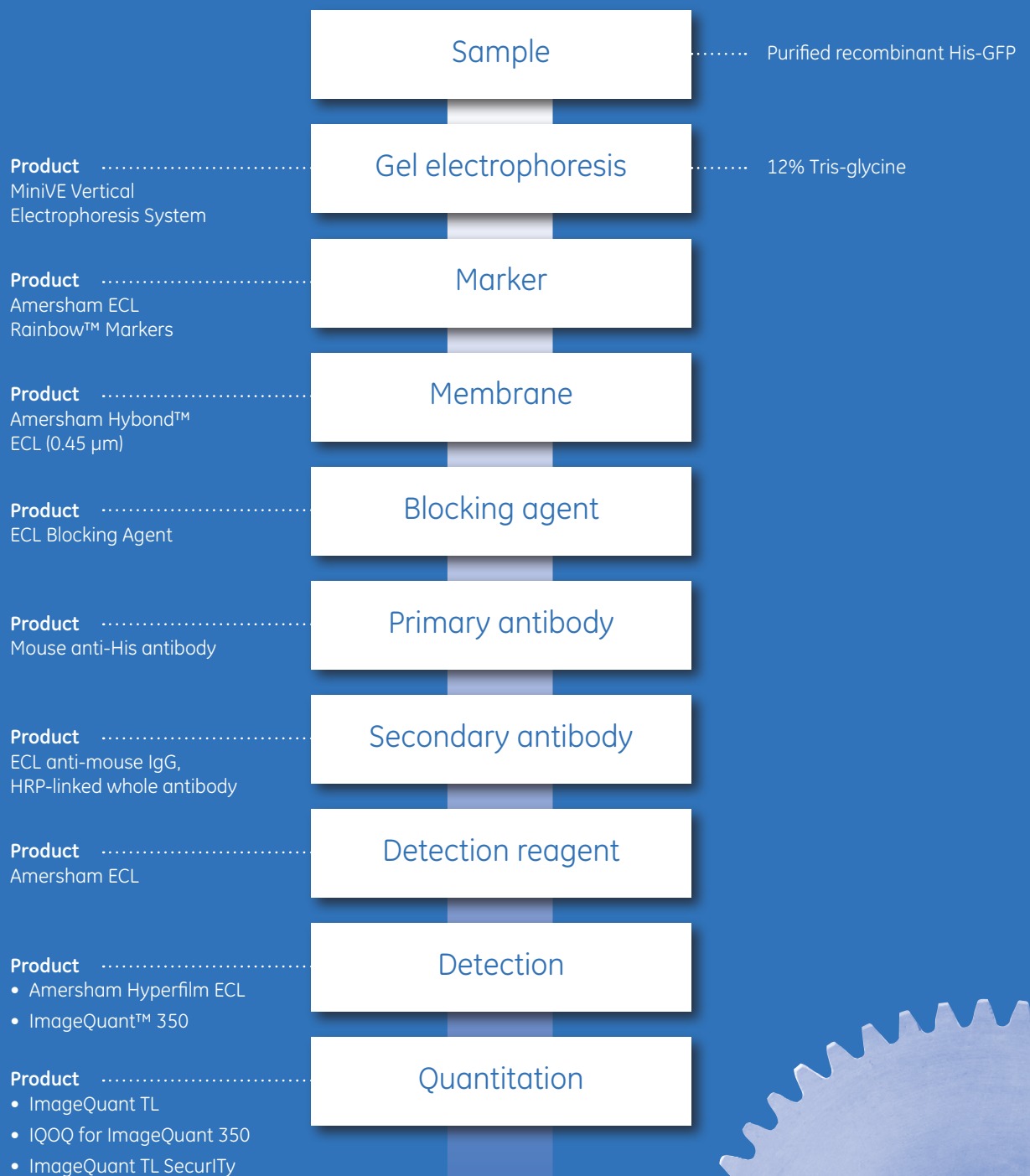
Some labs need a digital imaging solution with image audit control and qualified system installation. For these labs, we offer ImageQuant TL SecurITy software and IQOQ procedures for the ImageQuant 350 imager, the digital imaging system of choice for routine Western blotting.

ImageQuant TL SecurITy workflow



ImageQuant 350 imager





Versatile, quantitative Western blotting

When it is important to measure small differences in protein levels confidently, Amersham ECL Plus provides the necessary high sensitivity and dynamic range. With the possibility of choosing between chemiluminescent and chemifluorescent detection, using film or imagers, this is a flexible alternative for the precise detection of low abundant proteins.

Many cancers and other diseases are characterized by the inappropriate and sustained activation of specific molecules in signaling cascades. Analysis of these events requires the ability to quantitate both the expression and specific activation of the protein under scrutiny.

- *Precise and sensitive quantitation of proteins*
- *A familiar chemiluminescent signal for traditional imaging methods*
- *The stable chemifluorescent signal is useful for large experimental series*
- *Versatile imaging using film, a CCD camera or a laser scanner*

How to detect invisible changes in protein levels

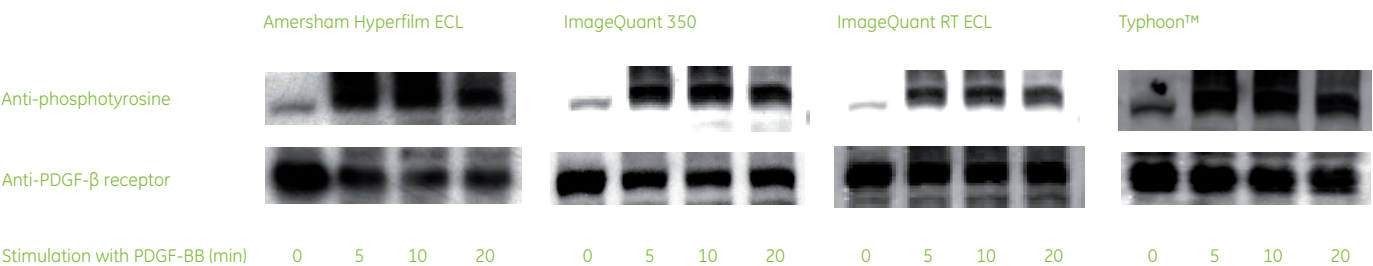
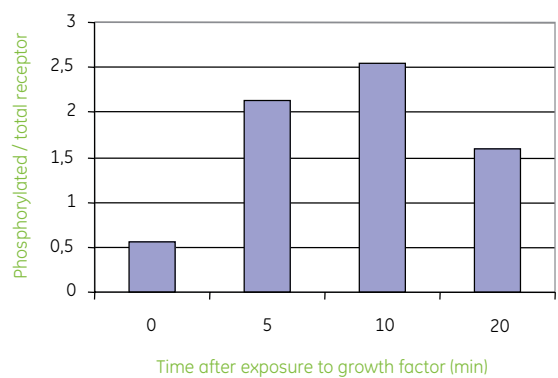
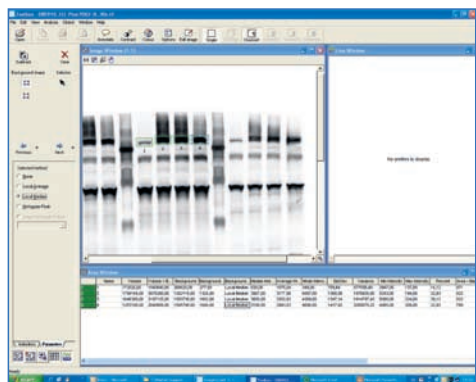
The dynamic profile of PDGF- β receptor phosphorylation was followed in cultured embryonic fibroblasts after exposure to the growth factor, PDGF-BB. Receptor phosphorylation was measured using a phosphotyrosine-specific antibody. After stripping and reprobing with an antibody to PDGF- β receptor, relative phosphorylation was calculated.

After only 5 min stimulation with PDGF-BB, tyrosine phosphorylation of PDGF- β receptors was greatly increased. At the same time point, however, total receptor levels were unchanged, indicating that the increase in signal was due specifically to receptor phosphorylation rather than an increase in receptor expression.

The broad dynamic range and high sensitivity of Amersham ECL Plus enabled detection and quantitation of these small differences in protein levels. Even within the 20 min time frame of the experiment, differences in tyrosine phosphorylation at each time point were accurately detected.

Signal precision across several orders of magnitude of concentration means that you can confidently quantitate weak and strong bands on a single image. In addition, the images can be captured optimally in a single automatic exposure, avoiding the potentially time consuming hit-or-miss trials necessary in exposing film.

ImageQuant TL analysis



Detection of PDGF- β receptor and tyrosine phosphorylated receptor using either chemiluminescence or chemifluorescence signals from Amersham ECL Plus demonstrates the versatility of this detection system

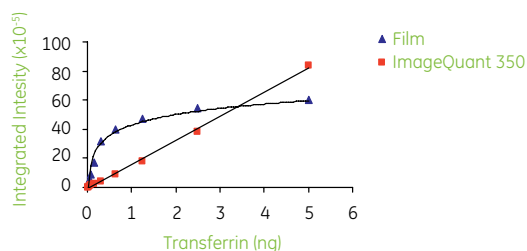
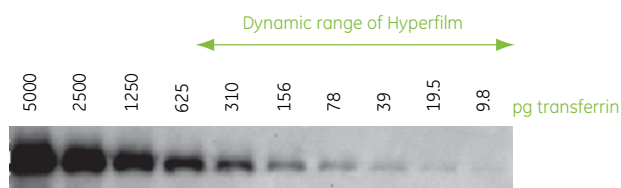
How to get better quantitation with an imager

ImageQuant 350 imager, ImageQuant TL and Amersham ECL Plus offer exceptional precision in protein detection and analysis across a wide dynamic range. Results were compared with those using Amersham Hyperfilm ECL.

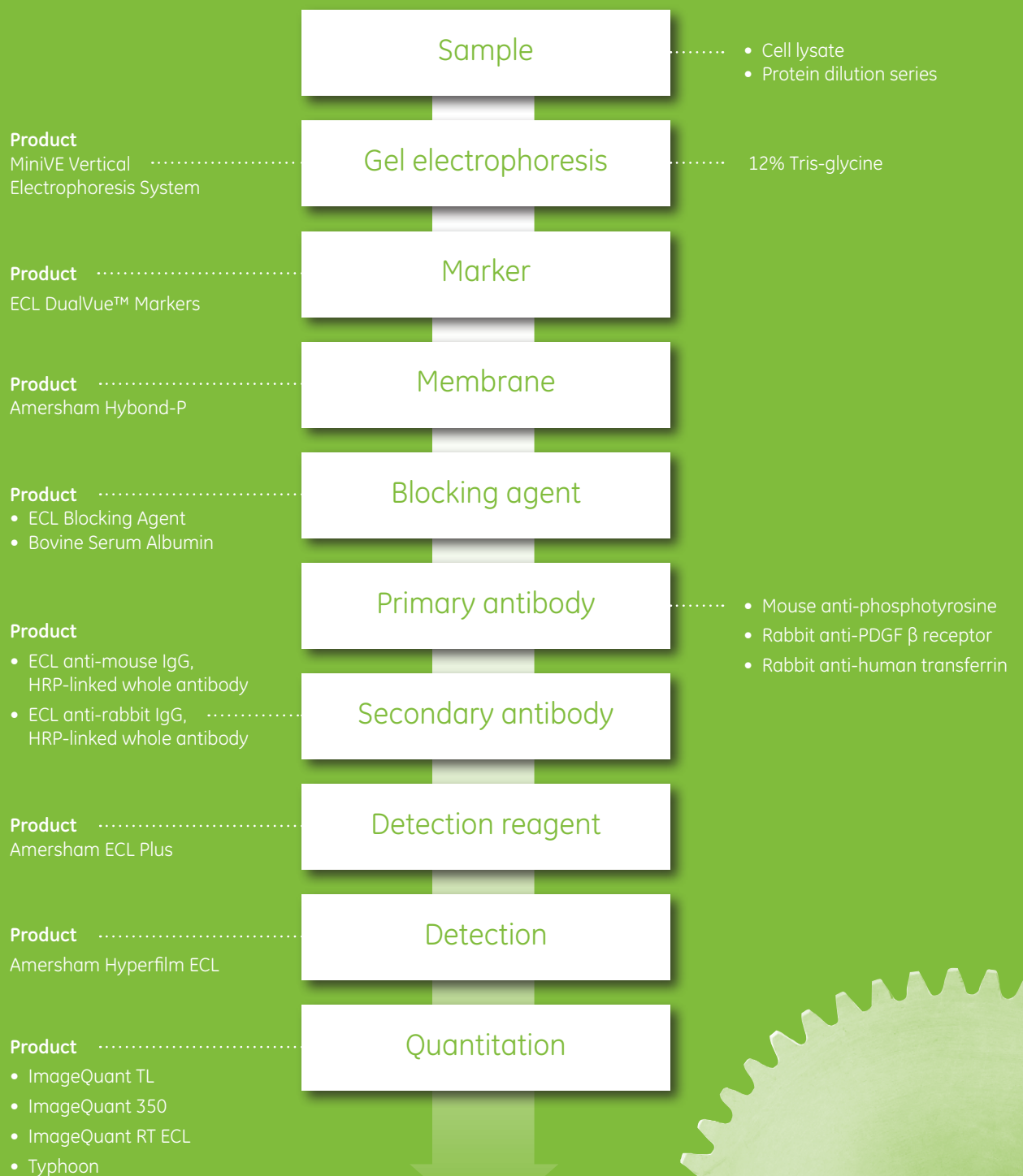
Although Amersham Hyperfilm ECL and ImageQuant 350 are equally sensitive, bands on Amersham Hyperfilm ECL became saturated by 625 pg of transferrin. Signal intensity on ImageQuant 350 is linear throughout the entire dynamic range tested, from 5 ng to 9.8 pg of transferrin. For even more sensitivity in an imaging system, ImageQuant RT ECL imager is the preferred solution.

Versatile chemiluminescence and chemifluorescence detection

Amersham ECL Plus offers the versatility of two detection technologies. Chemiluminescence, while sensitive, should be detected within 1 h after incubation with the ECL reagent as the signal deteriorates. Fluorescence, on the other hand, gives a stable and identical signal for at least 18 h, allowing a reliable comparison of results between blots, especially in a larger experimental series.



A twofold dilution series of transferrin, illustrating high sensitivity and broad dynamic range when using Amersham ECL Plus together with ImageQuant 350. Note that although similar sensitivity is achieved using film, signals approach saturation for the higher concentrations of transferrin, resulting in a much reduced dynamic range.



Efficient use of precious primary antibodies

What do you do when your primary antibody is very expensive or scarce, but must still be used at a sufficiently high concentration to maintain acceptable assay sensitivity? Pressure to minimize primary antibody consumption may lead to a compromise in the design of the assay, for example through the use of fewer controls.

With Amersham ECL Advance, highly diluted primary antibodies can be used in Western blot detection with no compromise in assay sensitivity or information content.

- *Equivalent sensitivity at a fraction of typical antibody consumption*
- *Precise quantitation of protein content*
- *Isoform resolution by 2-D Western blotting*

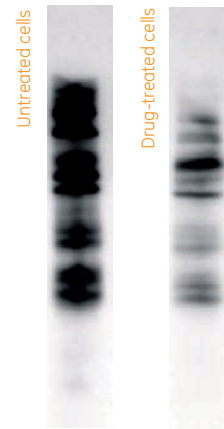
How to see more protein with less antibody

The proteome of many cancers is characterized by widespread phosphorylation, possibly indicating hyperactivation of signaling cascades. Drugs aimed at reducing phosphorylation either by inhibiting kinases or stimulating the activity of phosphatases may thus be promising therapeutic candidates.

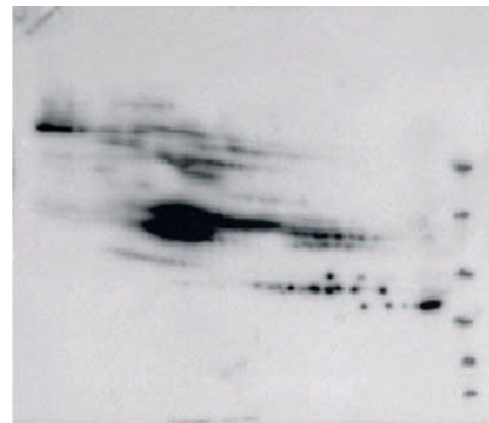
Variations in the tyrosine phosphorylation pattern were measured in cancer cells treated with a drug candidate and compared with untreated cells. The anti-phosphotyrosine antibody, 4G10, was used to probe membranes from both 1-D and 2-D gels.

Detection with Amersham ECL Advance showed that the proteome of treated cancer cells showed less extensive and less intense phosphorylation than the untreated control.

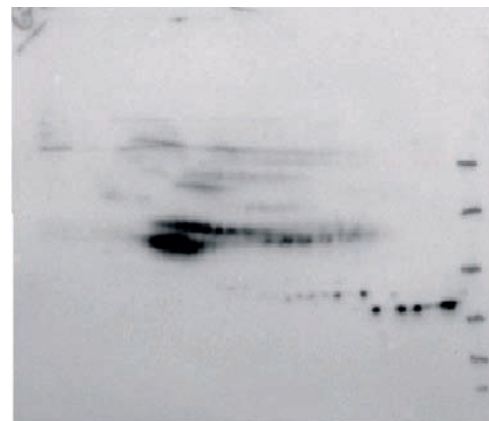
The resolution afforded by Amersham ECL Advance was more than sufficient to detect the minute changes in individual protein isoforms with highly diluted antibodies.



Untreated cells



Drug-treated cells

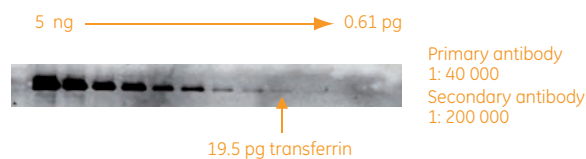


Comparison of lysates from treated and untreated cancer cells after 1-D and 2-D Western blotting and detection using Amersham ECL Advance. A proteome-wide reduction in phosphorylation of the treated cells is seen in both blots. Data courtesy of Dr Sara Lind, Rudbeck Laboratory, Uppsala, Sweden.

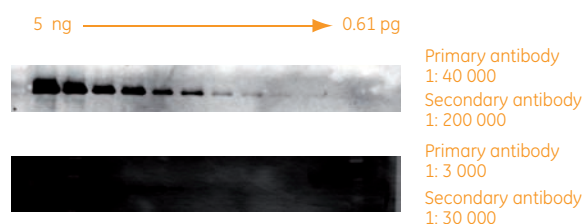
The secret of success with Amersham ECL Advance

You will only realize the benefit of Amersham ECL Advance when antibodies are highly diluted, typically to levels up to ten times more dilute than you are used to. To demonstrate this, a twofold dilution series of transferrin was prepared from 5 ng to 0.61 pg. Following electrophoresis and transfer, protein levels were precisely quantitated using primary antibodies diluted 1: 40 000 and secondary antibodies diluted 1: 200 000.

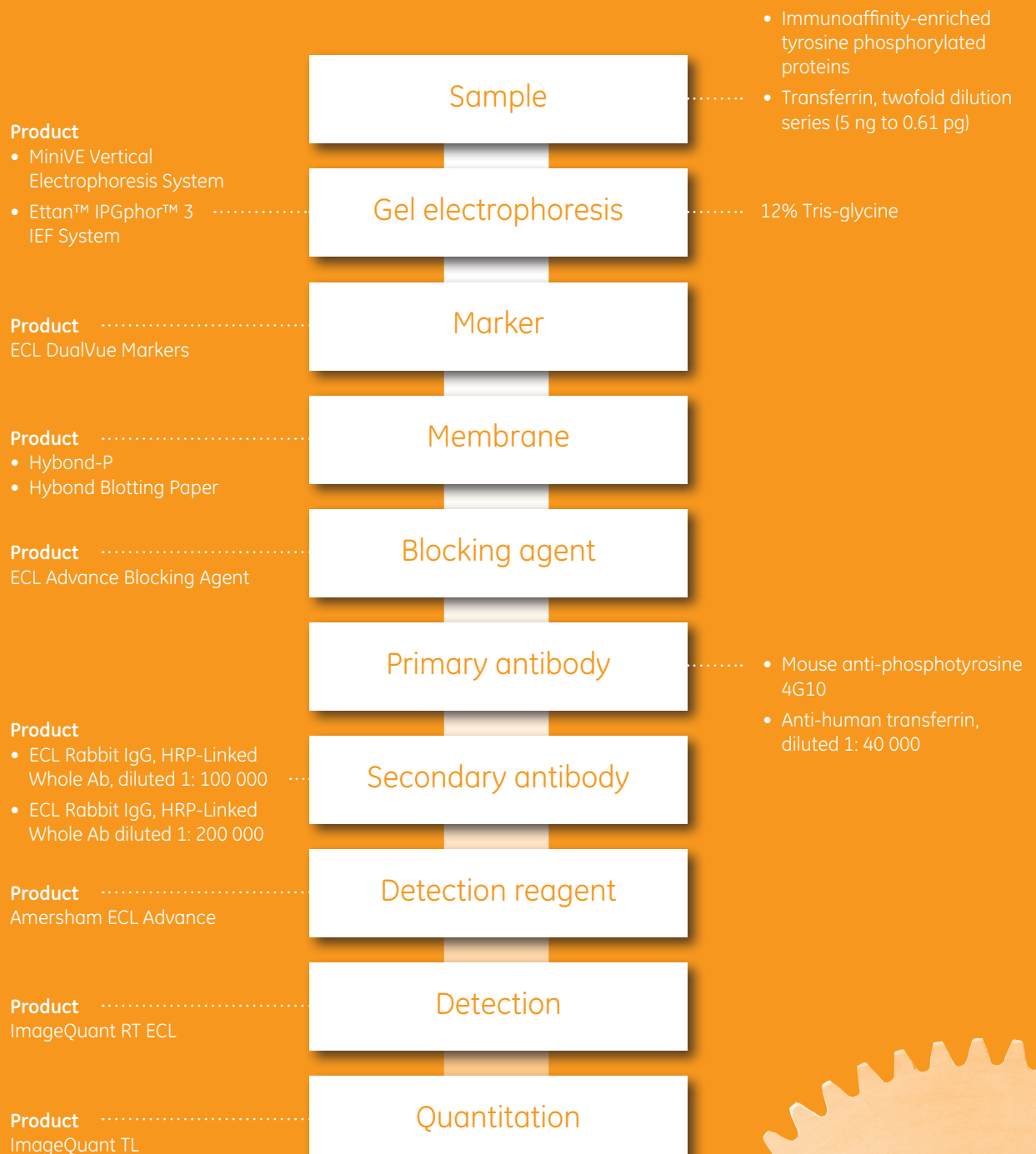
Using extremely dilute antibodies, detection and imaging with Amersham ECL Advance on ImageQuant RT ECL enabled precise quantitation down to picogram levels, a requirement for the detection of low abundance proteins.



A dilution series of transferrin, illustrating the high sensitivity of Amersham ECL Advance. Protein levels down to 19.5 pg (arrow) were precisely quantitated despite the use of extreme dilutions of primary and secondary antibodies. The signals were quantitated using ImageQuant RT ECL.



The upper blot shows results from a dilution series of transferrin using primary antibody diluted 1: 40 000 and secondary antibody diluted 1: 200 000. When the experiment was repeated using more typical dilutions of the same antibodies (primary at 1:3 000 and secondary at 1:30 000) the enhanced sensitivity of Amersham ECL Advance led to saturated signals in the entire blot.



Multiplex protein analysis

Quantitative, multiplex protein analysis by Western blotting

Imagine that you want to be able to quantitate minute changes in intracellular protein expression as well as post-translational modifications (PTMs).

And imagine that you can simultaneously detect and precisely quantitate several parent proteins and PTMs at the same time.

With fluorescence-based Amersham ECL Plex, all this is possible on one blot, in a single experiment.

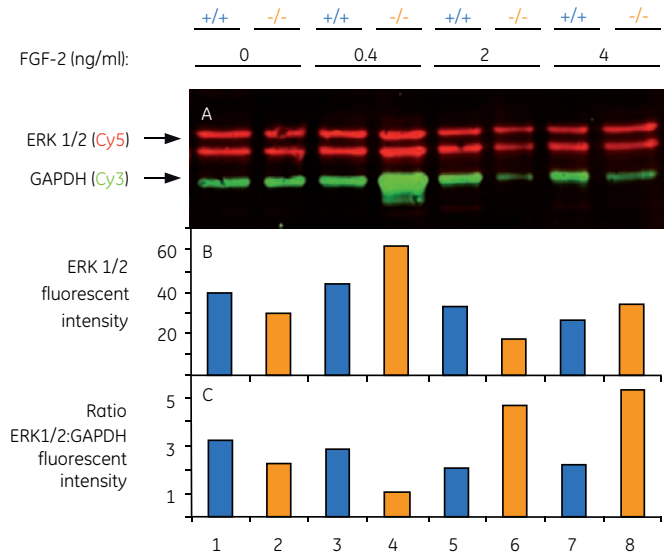
- *Targets of similar molecular weights, including PTMs, can be quantitated without compromising the results*
- *Normalize signals to that of an unregulated, endogenous internal standard protein for reliable quantitative results*
- *Simultaneously probe membranes with up to three primary antibodies*

The importance of good housekeeping

Comparisons of specific cellular protein levels or PTMs, for example after external stimulation, are only valid if the total amount of protein loaded on the gel is controlled. Although care may be taken to apply equal loads to lanes in a gel according to protein quantitation analysis, it is difficult to ensure. With Amersham ECL Plex, you can simultaneously measure more than one protein. This makes it possible to truly quantitate regulated proteins by comparison to stable endogenous internal standards, or “housekeeping” proteins. Each measurement can thereby be normalized to compensate for loading variations, without the risk of error compounded by membrane stripping and reprobing.

Lysates from wild type and enzyme knockout fibroblasts treated with FGF-2 were run on a gel. The membrane was simultaneously probed with anti-ERK1/2 and anti-GAPDH, followed by ECL Plex anti-mouse CyTM 5 and ECL Plex anti-rabbit Cy3.

Although protein quantitation analysis indicated that a similar amount of total protein was loaded in each lane, the intensity of the GAPDH signals clearly showed that this was not the case (panel A). Without relating to the GAPDH levels, no significant pattern of ERK1/2 corresponding to wild type and knock-out cells was seen (panel B). However, when normalized against GAPDH signals, ERK1/2 levels were shown to be increased in the knock-out cells in response to treatment with 2 and 4 ng/ml FGF-2 (lanes 6 and 8, panel C).



In this multiplex Western blot, primary antibodies to ERK1/2 and the housekeeping protein, GAPDH were simultaneously used to probe a membrane. Relating the signals to GAPDH signals indicates the amount of protein applied to each lane of the gel, allowing the true effect of FGF-2 treatment on ERK1/2 to be quantitated with precision and confidence. +/+ indicates wild type cells, -/- indicates knock-out cells.

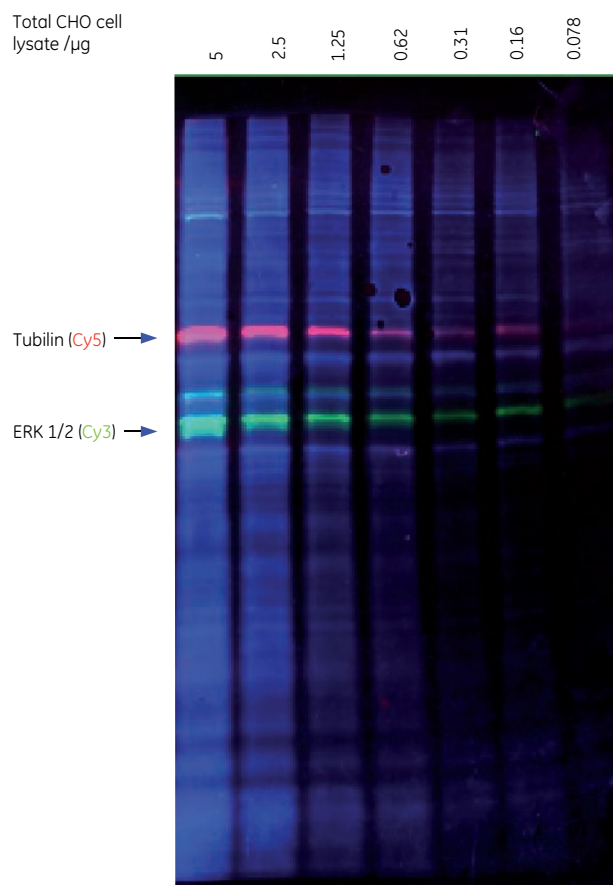
How to detect specific proteins normalized against total proteins

Another way to simultaneously measure changes in specific proteins while keeping an eye on total protein levels is to first stain the membrane with Deep Purple™ Total Protein Stain before multiplexing using Amersham ECL Plex to detect the proteins of interest. All the proteins present in a sample as well as specific proteins can be detected simultaneously, allowing dual detection as well as normalization.

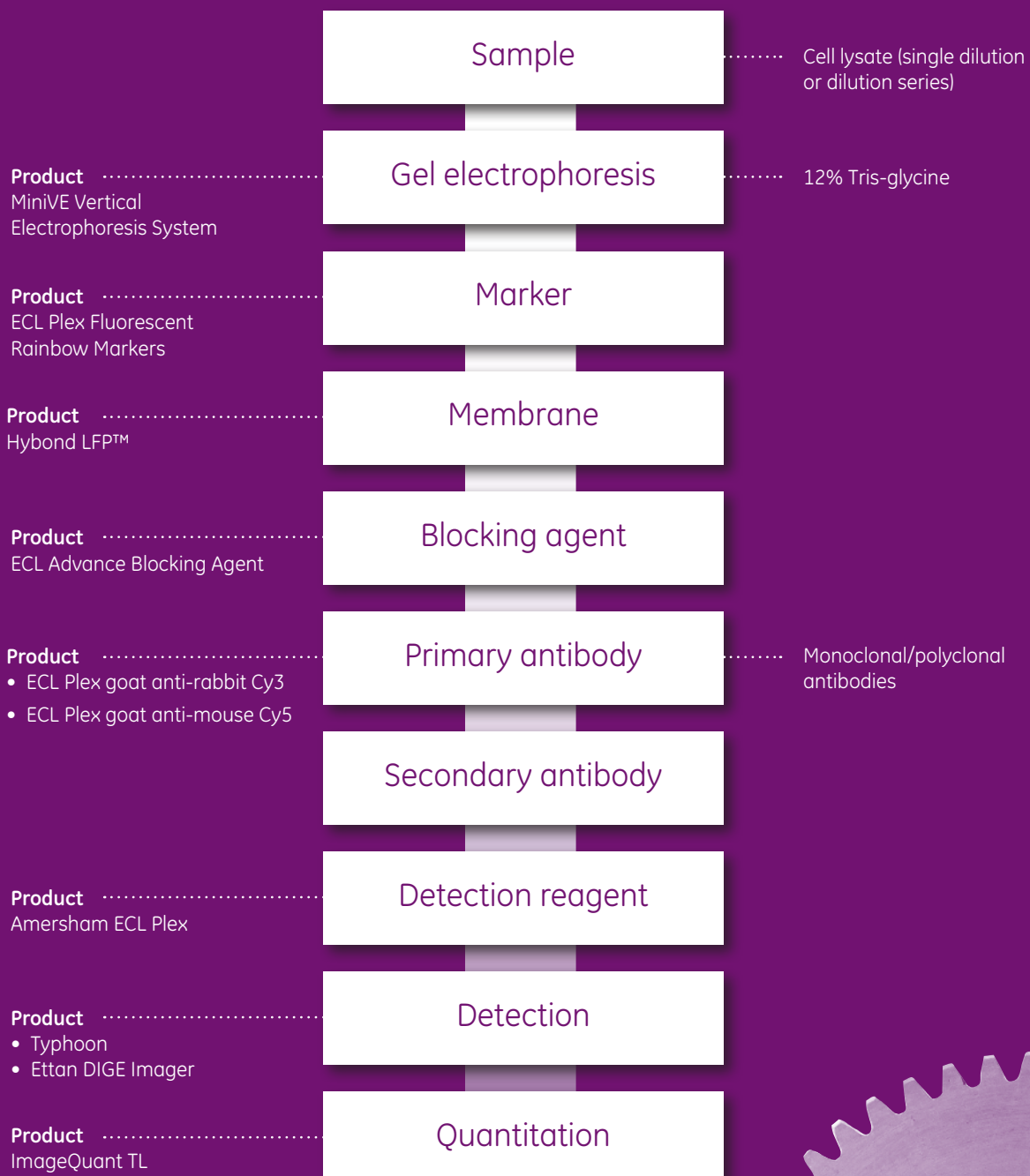
In this example, whole CHO cell lysates were run on a gel. After electrophoresis and transfer, membranes were stained with Deep Purple Total Protein Stain before probing with two specific primary antibodies, rabbit anti-ERK 1/2 and mouse anti- β -tubulin. Detection was by Amersham ECL Plex anti-mouse Cy5 and Amersham ECL Plex anti-rabbit Cy3.

Identical ECL Plex results were obtained with and without Deep Purple Total Protein Stain (not shown), demonstrating that Deep Purple does not affect the ECL Plex signals.

GE Healthcare offers solutions for multiplex imaging on either Typhoon or Ettan DIGE Imager.



A membrane stained first with Deep Purple Total Protein Stain followed by specific probes, anti-ERK 1/2 and anti- β -tubulin. Detection was by Amersham ECL Plex with species-specific secondary antibodies conjugated to Cy3 and Cy5.





Detection, using Amersham ECL Plex, of low abundance phosphorylated Akt protein and total Akt protein in human prostate cancer cells after stimulation with TGF- β . Despite the minimal change in molecular weight as a result of phosphorylation, the duplex capability of Amersham ECL Plex enables a clear distinction between the two forms of the protein. Note the complete absence of signal in the Cy3 channel for the sample treated with kinase inhibitor.

Data courtesy of Marene Landström, Ludwig Institute for Cancer Research, Uppsala, Sweden

How to detect PTMs of low abundance proteins

The multiplexing potential of Amersham ECL Plex was particularly useful in the following applications as first, it was desired to visualize an induced PTM simultaneously with the parent protein. In addition, as the protein of interest was predicted to be expressed at very low levels, it was important to avoid the sample loss and reduction of protein activity inherent in the aggressive process of membrane stripping.

Cell lysates from human prostate cancer cells stimulated with TGF- β were run on gels. An Amersham Hybond-LFP membrane was targeted with mouse anti-Akt or rabbit anti-phospho-Akt, followed by secondary ECL Plex anti-mouse Cy5 and ECL Plex anti-rabbit Cy3.

Tyrosine phosphorylation of Akt was seen after stimulation with TGF- β . The results demonstrate how multiplexing using Amersham ECL Plex enables two epitopes on a single protein to be identified and simultaneously quantitated on a single Western blot.

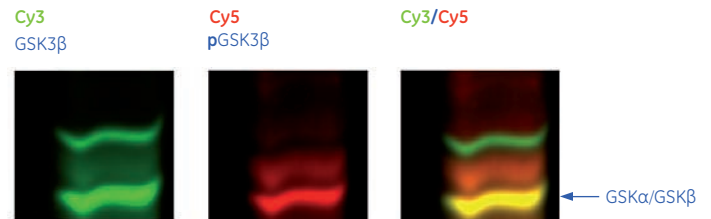
Quantitative duplex analysis of activated protein kinases

Glucogen synthase kinase 3 β (GSK3 β) and GSK3 β phosphorylated on serine residue 9 (pGSK3 β) were detected in human prostate cancer cells using Amersham ECL Plex. In addition, the larger α isoform of GSK was unspecifically detected by the primary antibody. Cell lysates were run on both 1-D and 2-D gels. Following electrophoresis and blotting, the membranes were probed with antibodies to GSK3 β and pGSK3 β followed by ECL Plex goat-anti-mouse IgG-Cy3 and ECL Plex goat-anti-rabbit IgG-Cy5.

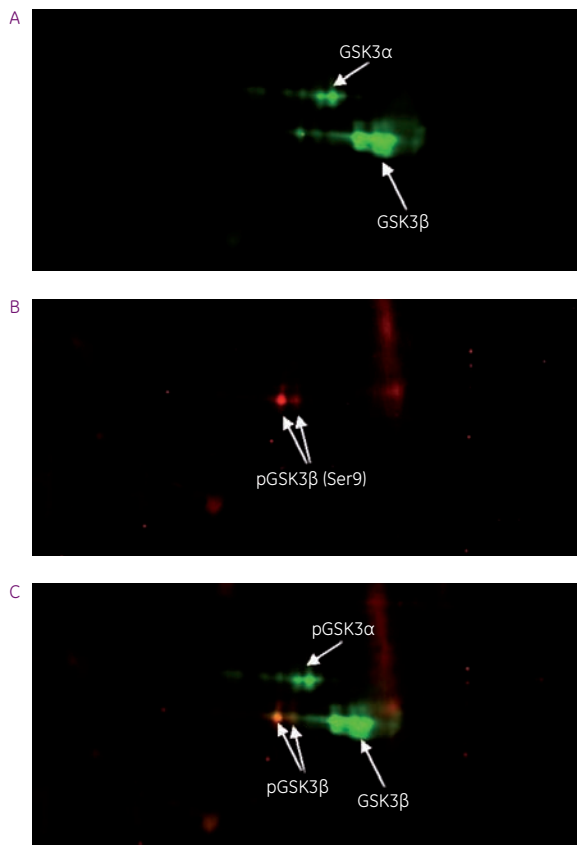
Each additional phosphate group added to GSK3 β induces a change in pI. With six potential phosphorylation sites, the precise phosphorylation status of GSK3 β can thus be detected on a 2-D gel. 2-D analysis shows that the M_r 48 000 band corresponding to pGSK3 β in the 1-D experiments resolved into at least five distinct isoforms, of which two were phosphorylated on serine 9, as recognized by the anti-pGSK3 β primary antibody.

1-D Western blotting showed that serine 9-phosphorylated GSK3 β was present in PC-3U cells. When applied to 2-D gel electrophoresis, Amersham ECL Plex was used to resolve the PTM states of GSK3 β into five alternative isoforms.

The results revealed that only the 2 most extensively phosphorylated isoforms of GSK3 β were phosphorylated on serine 9.



Images resulting from scanning with the green laser exciting (Cy3) for GSK3 β , the red laser exciting (Cy5) for pGSK3 β , and the overlay of scans (Cy3/Cy5). The yellow color of the GSK β band shows that the signals from the two primary antibodies overlap: the band contains phosphorylated GSK3 β .



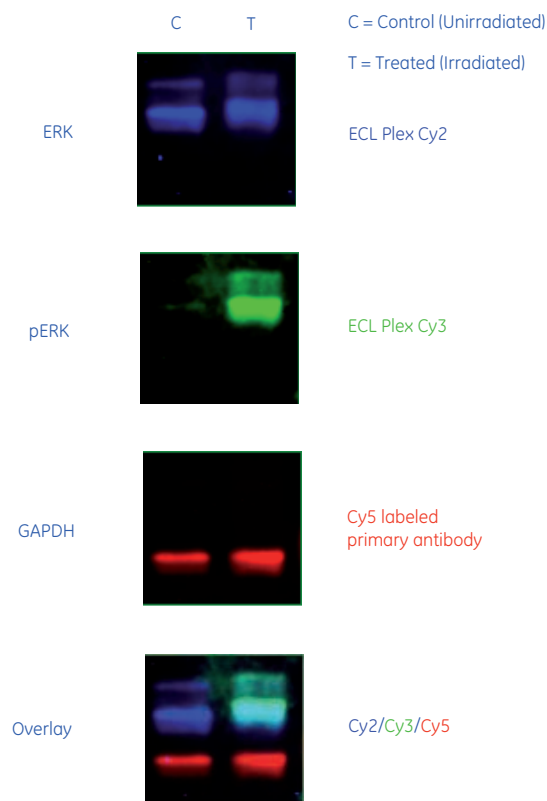
(A) GSK3 β and GSK α (unspecific) detected using Amersham ECL Plex goat-anti-mouse IgG-Cy3 (green); (B) pGSK3 β detected using ECL Plex goat-anti-rabbit IgG-Cy5 (red); (C) Overlay of (A) and (B).

Quantitative triplex analysis

Amersham ECL Plex has now been further developed to bring protein analysis into the realm of quantitative triplex analysis.

To observe the effect of irradiation on selected intracellular proteins, three proteins; ERK, phosphorylated ERK (pERK) and the housekeeping protein, GAPDH, were selected for simultaneous analysis using Amersham ECL Plex, where either primary or secondary antibodies were labeled with fluorescent CyDye™ fluors. Since CyDye fluors emit discrete signals with minimal cross-talk, the three proteins could be differentially labeled and identified on a single blot.

Triplex analysis of the cell lysate enables simultaneous quantitation of two targets while, in the same experiment, relating signals to those of a house keeping protein to control for sample load. This is very useful in cases where the pool of protein as well as the PTM of interest varies.



Quantitative triplex analysis of a cell lysate of HeLa cells labeled with antibodies to ERK, pERK and GAPDH.

Product

- 1-D, MiniVE Vertical Electrophoresis Unit
- 2-D, Immobiline™ DryStrip pH 7-11
- Ettan IPGphor 3 IEF System

Sample

Cell lysate (single dilution or dilution series)

Gel electrophoresis

12% Tris-glycine

Product

ECL Plex Fluorescent Rainbow Markers

Marker

Product

Hybond LFP

Membrane

Product

ECL Advance Blocking Agent

Blocking agent

Product

Cy5 Ab Labeling Kit

Primary antibody

Monoclonal/polyclonal antibodies

Product

- ECL Plex goat anti-rabbit Cy3 & Cy5
- ECL Plex goat anti-mouse Cy3 & Cy5
- ECL Plex goat anti-rabbit Cy2

Secondary antibody

Product

Amersham ECL Plex

Detection reagent

Product

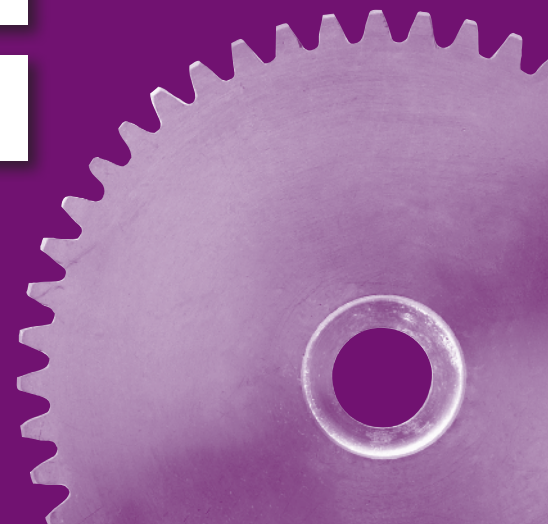
- Typhoon
- Ettan DIGE Imager

Detection

Product

ImageQuant TL

Quantitation



Amersham ECL protein detection - how it works

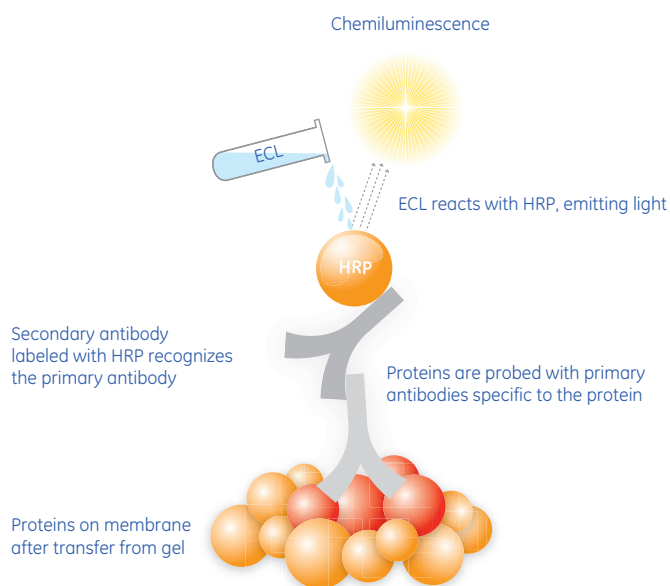
Amersham ECL and Amersham ECL Advance

Amersham enhanced chemiluminescence (Amersham ECL) was the world's first commercially available nonradioactive means of detecting proteins. Amersham ECL enables detection of minute quantities of the protein based on a signal that is proportional to the amount of protein, making it suitable for detecting proteins over a wide range of concentrations.

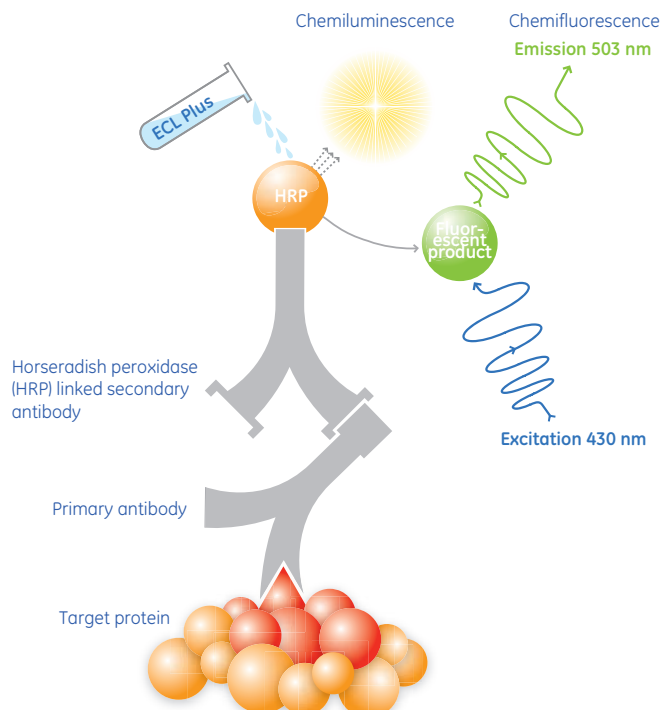
Amersham ECL Plus

Amersham ECL Plus is the only multifunctional detection system for Western blotting. It produces both a chemiluminescent as well as a chemifluorescent signal. Amersham ECL Plus is versatile in that its chemifluorescent signal can be detected by Typhoon imager and Ettan DIGE Imager, and its chemiluminescent signal can be detected on ImageQuant 350 and ImageQuant RT ECL imagers as well as Hyperfilm ECL.

The possibility to generate a quantitative chemifluorescent signal makes ECL Plus a good complement to ECL Plex, since the same imager can be used for both detection systems.



Horseradish peroxidase (HRP) is tethered to the protein of interest through a secondary antibody that recognizes the primary antibody for the protein. This enzyme catalyzes the conversion of the ECL substrate into a sensitized reagent, which on further oxidation produces an excited state that emits light when it decays.

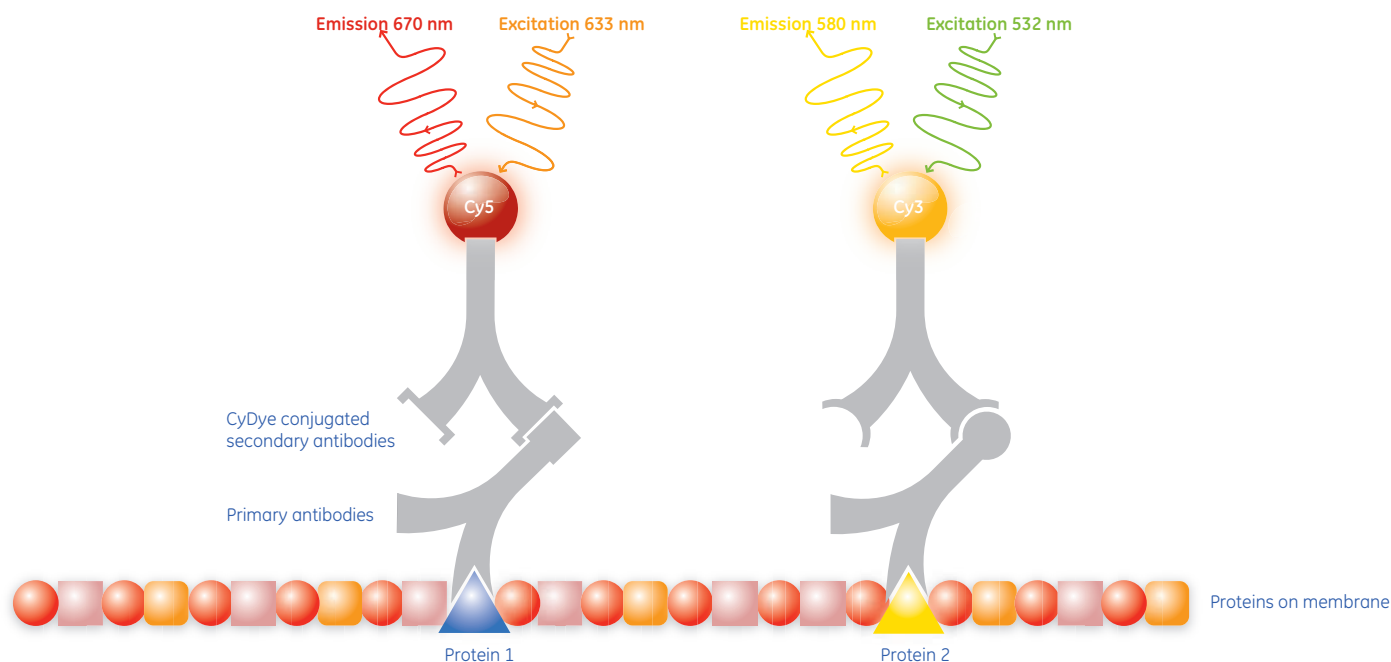


The reaction between ECL Plus and HRP generates a chemiluminescent emission. Simultaneously, a fluorescent by-product is produced. The fluorescent product is stable for at least 18 h and can be measured by a fluorescence imager with an excitation wavelength near 430 nm. The resultant fluorescent emission peak is at 503 nm.

Amersham ECL Plex

Fluorescent detection enables multiplex detection - simultaneous detection of more than one protein target. Two primary antibodies that are raised in different species are used to recognize the two protein targets. The fluorescent emission signals are captured by a multichannel fluorescent imager such as Typhoon

or Ettan DIGE Imager. A third secondary antibody can be labeled with Cy2 for triplex imaging applications. Amersham ECL Plex has the added benefits of fewer handling steps and signal longevity. Signals are stable for up to three months, enabling researchers to compare data across several experiments.



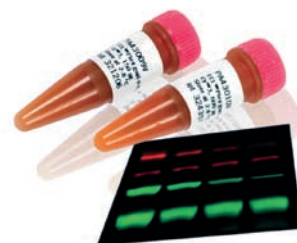
Primary antibodies against two proteins are recognized by species-specific secondary antibodies conjugated to the fluorescent dyes Cy3 and Cy5. Detection by direct fluorescence reduces the number of processing and imaging steps, which saves time and reduces errors in quantitation.

How to choose the right product

Amersham ECL detection systems

Amersham ECL Western Blotting Systems from GE Healthcare have been delivering sensitivity, speed, and versatility since 1990. Today, the world's first chemiluminescent reagents continue to be the most widely used and have established a well-earned reputation for excellence you can rely on, day in, day out. Amersham ECL, Amersham ECL Plus, and Amersham ECL Advance are now complemented by the Amersham ECL Plex fluorescent system, expanding your options even further. ECL Plex brings multiplex detection to Western blotting, enabling a faster detection process, normalization to internal loading standards for better quantitation, and accurate comparison of protein targets without the need to strip and reprobe the membrane.

Amersham ECL Plex



Amersham ECL



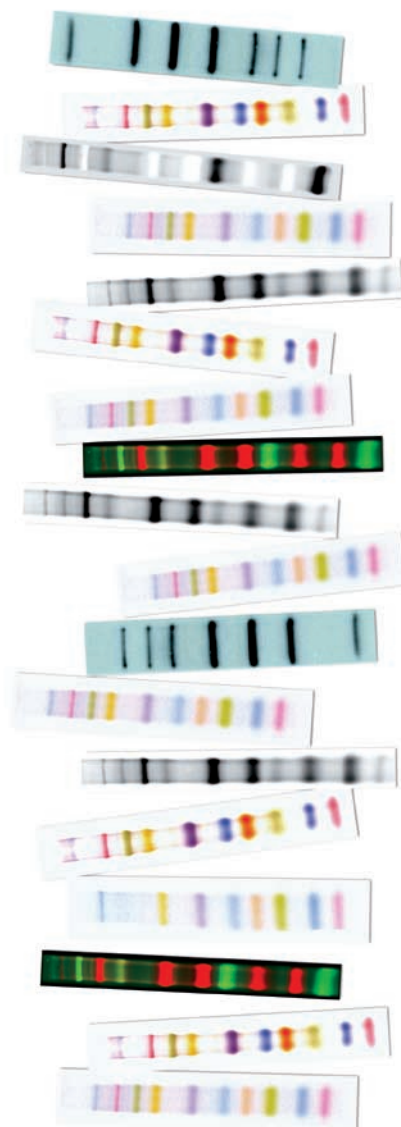
Rainbow Markers

Differentiating proteins with similar molecular weights requires clear protein standards. Rely on the proven performance of Amersham Rainbow Markers, redeveloped for even better resolution.

All these markers are good for confirming transfer and orientation (as the colored bands transfer to the membrane) as well as determination of molecular weight.

- Rainbow Markers, available as full-range (M_r 12 000 to 225 000, ten proteins), high-range (M_r 12 000 to 225 000, eight proteins), and low-range (M_r 3 500 to 38 000). Rainbow Markers are suitable for Amersham ECL, Amersham ECL Plus and Amersham ECL Advance. Sharp and strong colored bands appear on the gel during the run to control the progress of the electrophoresis.
- ECL Plex Fluorescent Rainbow Markers for quantitative Western blots, optimized for use with the Amersham ECL Plex system. The markers are specially optimized to provide visible marker bands on gels and membranes, as well as fluorescent images using Cy3 and Cy5 channels.
- ECL DualVue Markers, optimized for use with Amersham ECL, Amersham ECL Plus, and Amersham ECL Advance are the optimal positive controls for your Western blot. The colored bands, which are visible both on the gel and on the membrane after transfer, serve to indicate the progress of the electrophoresis, confirm protein transfer to the membrane, and help the user orientate the blot. In addition, the bands are also detected with the chemiluminescent substrates, appearing on the film or digital image and thus serve as a control for the detection system.

Rainbow Markers



How to choose the right product

Membranes

- Amersham Hybond-ECL is a nitrocellulose membrane compatible with all chemiluminescent Western blotting substrates. It is recommended for many total protein staining protocols.
- Amersham Hybond-P is a PVDF membrane suitable for all chemiluminescent Western blotting kits. It is a robust and durable membrane suitable for stripping and re-probing.
- Amersham Hybond-LFP is a low fluorescent PVDF membrane. It is optimized for use with Amersham ECL Plex and it can also be used with Amersham ECL Plus.

Amersham Hybond-ECL



Amersham Hybond-LFP



Transfer

- Wet transfer (TE 62), the most commonly used transfer method, is especially efficient for large proteins
- Semi-dry transfer (TE 70, TE 77), is faster than wet transfer and consumes less buffer. Semi-dry transfer works well for most proteins but transfer may be less efficient for large proteins. It also reduces sensitivity for very low abundance proteins.

ECL semi-dry blotter transfer unit



Blocking agents

Unwanted binding and the resulting background caused by the primary antibody can be minimized by choosing the best blocking agent. Compatibility of the blocking agent with the detection system is important as it will affect background.

- ECL Membrane Blocking Agent, is recommended for use with Amersham ECL and Amersham ECL Plus
- ECL Advance Blocking Agent is effective at reducing unspecific signals. It is developed for Amersham ECL Advance and it is also recommended for Amersham ECL Plex due to its low fluorescence
- BSA Blocking Agent is a generic blocking agent, compatible with all kits. It is particularly suitable for Amersham ECL Plex detection due to its low fluorescence

ECL Advance Blocking Agent



Imagers and film

The versatility of Typhoon variable mode imagers - filmless phosphor imaging, multiplex fluorescence imaging of ECL Plex, and chemifluorescence imaging of ECL Plus - enables you to select the most suitable imaging technique for a particular experiment.

The Ettan DIGE Imager is based on innovative scanning technology for fluorescence imaging with a novel, progressive scanning CCD system. It is suitable for a wide range of fluorescent probes, including ECL Plus, ECL Plex, Deep Purple, and 2-D DIGE CyDye labeling.

The ImageQuant imager range, including ImageQuant 350 and ImageQuant RT ECL, are flexible systems that cover a range of monochromatic imaging applications including chemiluminescence imaging of Amersham ECL, ECL Plus and ECL Advance, monochromatic fluorescence, and densitometry. These CCD-based imagers provide an affordable option for protein detection with genuine benefits, including better quantitation than film without sacrificing sensitivity, publication grade data, and image and data archiving.

Amersham Hyperfilm ECL is a trusted product for reliable qualitative chemiluminescent detection in Western blots.

ECL Network

The ECL Network gathers years of expertise of experimental design in one place, with a user forum supported by GE scientists and engineers. For technical help, tips, and best practices, register at www.eclnetwork.com

Typhoon



ImageQuant imagers



Amersham Hyperfilm ECL



ECL Network



References

Signal transduction/ secondary messenger regulation

Nyunoya, T. *et al.* Macrophages survive hyperoxia via prolonged ERK activation due to phosphatase down-regulation. *J. Biol. Chem.* **280**, 26295-26302 (2005).

Products used:

Hybond ECL nitrocellulose membranes, Amersham ECL and Amersham ECL Plus Western Blotting detection systems

Cell surface receptor: ligand interactions

Gilchrist, R.B. *et al.* Immunoneutralization of growth differentiation factor 9 reveals it partially accounts for mouse oocyte mitogenic activity. *Biol. Reprod.* **71**, 732-739 (2004).

Products used:

Amersham ECL and Amersham ECL Advance Western Blotting detection systems

Hailey, J. *et al.* Neutralizing anti-insulin-like growth factor receptor 1 antibodies inhibit receptor function and induce receptor degradation in tumor cells. *Mol. Cancer. Ther.* **1**, 1349-1353 (2002).

Product used:

Amersham ECL Western Blotting detection system

Kohno, T. *et al.* N-glycans of sphingosine 1-phosphate receptor Edg-1 regulate ligand-induced receptor internalization. *FASEB J.* **16**, 983-992 (2002).

Products used:

Amersham ECL and Amersham ECL Plus Western Blotting detection systems

Woszczek, G. *et al.* IFN-gamma induces cysteinyl leukotriene receptor 2 expression and enhances the responsiveness of human endothelial cells to cysteinyl leukotrienes. *J. Immunol.* **178**, 5262-5270 (2007).

Products used:

*Amersham ECL Advance Western Blotting Detection Kit
Amersham ECL Advance Blocking Agent*

Modulation of protein expression

Gavin, B.A. *et al.* Accelerated accumulation of misfolded prion protein and spongiform degeneration in a *Drosophila* model of Gerstmann-Sträussler-Scheinker syndrome. *J. Neurosci.* **26**, 12408-12414 (2006).

Product used:

Amersham ECL Advance Western Blotting Detection Kit

Pellizzaro, C. *et al.* Modulation of angiogenesis-related proteins synthesis by sodium butyrate in colon cancer cell line HT29. *Carcinogenesis* **23**, 735-740 (2002).

Products used:

Hybond ECL nitrocellulose membranes, Hyperfilm ECL and Amersham ECL Western Blotting detection systems

Yu, C.X. *et al.* Redox regulation of PTEN by S-nitrosothiols. *Mol. Pharmacol.* **68**, 847-854 (2005).

Product used:

Amersham ECL Plus Western Blotting detection reagent

Proteomics

Alm, H. *et al.* Proteomic evaluation of neonatal exposure to 2,2,4,4,5-pentabromodiphenyl ether. *Environ. Health Perspect.* **114**, 254-259 (2006).

Products used:

*Amersham ECL Plex Western Blotting Detection System
CyDye DIGE Fluor Minimal labeling kit
Immobiline DryStrip Gels for IEF, 24 cm
IPG Buffer
Ettan IPGphor 3 Isoelectric Focusing Unit
Ettan DALTsix Electrophoresis Unit
Typhoon 9400*

Donoghue, P.M. *et al.* CyDye immunoblotting for proteomics: co-detection of specific immunoreactive and total protein profiles. *Proteomics* **6**, 6400-6404 (2006).

Products used:

Amersham ECL Plex Western Blotting Detection System

Ordering information

Products	Quantity	Code no.	Products	Quantity	Code no.
Sample preparation			Amersham Markers		
Mammalian Protein Extraction Buffer	1 x for 500 ml	28-9412-79	Low-Range Rainbow Molecular Weight Markers	250 µl	RPN755E
Yeast Protein Extraction Buffer Kit	1	28-9440-45	High-Range Rainbow Molecular Weight Markers	250 µl	RPN756E
Vivaspin™ sample concentrators with 30 000 molecular weight cut off* (MWCO)			Full-Range Rainbow Molecular Weight Markers	250 µl	RPN800E
2-D Quant Kit	500 assays	80-6483-56	ECL DualVue Western Blotting Markers	1 pack (25 loadings)	RPN810
Vivaspin 500	25 x 500 µl	28-9322-35	ECL Plex Fluorescent Rainbow Markers	120 µl	RPN850E
Vivaspin 2	25 x 2 ml	28-9322-48	ECL Plex Fluorescent Rainbow Markers	500 µl	RPN851E
Vivaspin 6	25 x 6 ml	28-9323-17	Amersham ECL Western Blotting Molecular Weight Markers	1 pack (25 loadings)	RPN2107
Vivaspin 20	12 x 20 ml	28-9323-61			
* Other MWCO (3 000, 5 000, 10 000, 50 000 and 100 000) are available for all Vivaspin column volumes.			Blotting Membranes		
Gel electrophoresis and transfer equipment			Hybond ECL (20 x 20 cm)	10 sheets	RPN2020D
ECL Semidry Blotters			Hybond-C Extra (20 x 20 cm)	10 sheets	RPN2020E
TE 70 Semi-Dry Transfer Unit, 14 x 16 cm	1	80-6210-34	Hybond-P (20 x 20 cm)	10 sheets	RPN2020F
TE 70 PWR Semi-Dry Transfer Unit, 14 x 16 cm†	1	11-0013-41	Hybond-LFP (20 x 20 cm)	10 sheets	RPN2020LFP
TE 77 Semi-Dry Transfer Unit, 21 x 26 cm	1	80-6211-86	Hybond-LFP (20 x 20 cm)	3 sheets	RPN2020LFP3
TE 77 PWR Semi-Dry Transfer Unit, 21 x 26 cm†	1	11-0013-42	Hybond Blotting Paper (20 x 20 cm)	100 sheets	RPN6101M
TE 62 Transfer Cooled Unit	1	80-6209-58			
Blotting Equipment					
ECL Multiprobe	1	11-0033-95			
ECL Multiprobe XL	1	11-0033-96			
Electrophoresis Systems					
miniVE Vertical Electrophoresis System	1	80-6418-77			
miniVE Blot Module	1	80-6418-96			
SE 260 Mini-Vertical Unit for two slab gels	1	80-6149-35			
SE 600 Ruby Standard Dual Cooled Vertical Unit	1	80-6479-57			
Power Supplies					
EPS 301 Power Supply	1	18-1130-01			
EPS 2A200 Power Supply		80-6406-99			
† incl. Power Supply					

Ordering information

Products	Quantity	Code no.
Blocking Reagents		
ECL Advance Blocking Reagent	20 g	RPN418
ECL Blocking Agent	1	RPN2125
Bovine Serum Albumin	25 g	RPN412

Primary Antibodies

Anti-His Antibody	170 µl	27-4710-01
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Amersham CyDye Antibody Labeling Kits

Cy2 Ab Labeling Kit	1 kit	PA32000
Cy2 mAb Labeling Kit	1 kit	PA32001
Cy3 Ab Labeling Kit	1 kit	PA33000
Cy3 mAb Labeling Kit	1 kit	PA33001
Cy5 Ab Labeling Kit	1 kit	PA35000
Cy5 mAb Labeling Kit	1 kit	PA35001

Amersham ECL HRP-Linked Secondary Antibodies

ECL Mouse IgG, HRP-Linked Whole Ab (from sheep)	100 µl	NA931-100UL
ECL Mouse IgG, HRP-Linked Whole Ab (from sheep)	1 ml	NA931-1ML
ECL Human IgG, HRP-Linked Whole Ab (from sheep)	1 ml	NA933-1ML
ECL Rabbit IgG, HRP-Linked Whole Ab (from donkey)	100 µl	NA934-100UL
ECL Rabbit IgG, HRP-Linked Whole Ab (from donkey)	1 ml	NA934-1ML
ECL Rat IgG, HRP-Linked Whole Antibody (from goat)	1 ml	NA935
ECL Mouse IgG, HRP-Linked F(ab') ₂ Fragment (from sheep)	1 ml	NA9310-1ML
ECL Rabbit IgG, HRP-Linked F(ab') ₂ Fragment (from donkey)	1 ml	NA9340-1ML
ECL Rat IgG, HRP-Linked F(ab') ₂ Fragment (from goat)	1 ml	NA9350

Products	Quantity	Code no.
Kits and Reagent Packs		
Amersham ECL Western Blotting Detection Reagents for 4000 cm ² membrane		RPN2106
Amersham ECL Western Blotting System	1 kit	RPN2108
Amersham ECL Western Blotting Detection Reagents for 1000 cm ² membrane		RPN2109
Amersham ECL Western Blotting Detection Reagents for 6000 cm ² membrane		RPN2134
Amersham ECL Plus Western Blotting Reagent Pack	1	RPN2124
Amersham ECL Plus Western Blotting Detection Reagents for 1000 cm ² membrane		RPN2132
Amersham ECL Plus Western Blotting Detection Reagents for 3000 cm ² membrane		RPN2133
Amersham ECL Advance Western Blotting Detection Kit for 1000 cm ² membrane		RPN2135

Products	Quantity	Code no.
Amersham ECL Plex CyDye conjugated Antibodies		
Amersham ECL Plex Western Blotting Combination Pack (Cy3, Cy5, Hybond ECL)	1	RPN998
Amersham ECL Plex Western Blotting Combination Pack (Cy3, Cy5, Hybond LFP) for two slab gels	1	RPN999
ECL Plex goat- α -rabbit IgG-Cy3, for 1000 cm ² membrane area	150 μ g	28-9011-06
ECL Plex goat- α -rabbit IgG-Cy3, for 4000 cm ² membrane area	600 μ g	28-9011-07
ECL Plex goat- α -mouse IgG-Cy2, for 1000 cm ² membrane area	150 μ g	28-9011-08
ECL Plex goat- α -mouse IgG-Cy2, for 4000 cm ² membrane area	600 μ g	28-9011-09
ECL Plex goat- α -rabbit IgG-Cy2, for 1000 cm ² membrane area	150 μ g	28-9011-10
ECL Plex goat- α -rabbit IgG-Cy2, for 4000 cm ² membrane area	600 μ g	28-9011-11
ECL Plex goat- α -mouse IgG-Cy3, for 1000 cm ² membrane area	150 μ g	PA43009
ECL Plex goat- α -mouse IgG-Cy3, for 4000 cm ² membrane area	600 μ g	PA43010
ECL Plex goat- α -mouse IgG-Cy5, for 1000 cm ² membrane area	150 μ g	PA45009
ECL Plex goat- α -mouse IgG-Cy5, for 4000 cm ² membrane area	600 μ g	PA45010
ECL Plex goat- α -rabbit IgG-Cy5, for 1000 cm ² membrane area	150 μ g	PA45011
ECL Plex goat- α -rabbit IgG-Cy5, for 4000 cm ² membrane area	600 μ g	PA45012
Protein Staining Reagents		
Deep Purple Total Protein Stain	5 ml	RPN6305
Deep Purple Total Protein Stain	25 ml	RPN6306

Products	Quantity	Code no.
Autoradiography Films		
Amersham Hyperfilm ECL (5 x 7 inches)	50 sheets	28-9068-35
Amersham Hyperfilm ECL (18 x 24 cm)	50 sheets	28-9068-36
Amersham Hyperfilm ECL (18 x 24 cm)	100 sheets	28-9068-37
Amersham Hyperfilm ECL (8 x 10 inches)	50 sheets	28-9068-38
Amersham Hyperfilm ECL (8 x 10 inches)	100 sheets	28-9068-39
Amersham Hyperfilm ECL (24 x 30 cm)	50 sheets	28-9068-40
Amersham Hyperfilm ECL (35 x 43 cm)	50 sheets	28-9068-41
Imaging Systems		
<i>Typhoon Variable Mode Imagers</i>		
Typhoon Trio™	1	63-0055-87
Typhoon Trio+	1	63-0055-89
<i>Ettan DIGE Imager</i>		
Ettan DIGE Imager, including installation kit	1	63-0056-42
<i>ImageQuant Imagers</i>		
ImageQuant RT ECL	1	28-9043-15
<i>ImageQuant 350</i>		
ImageQuant 350 Standard	1	28-9261-75
ImageQuant 350 with Motorized Fixed Lens		28-9272-95
ImageQuant 350 Standalone (without lenses)		28-9383-46
Software and Accessories		
<i>CD and getting started:</i>		
ImageQuant TL 7.0 and IQTL SecurITy 8.0 Software package (with Getting Started Guide)		28-9380-94
<i>Licenses for ImageQuant TL only:</i>		
ImageQuant TL, single user license		28-9236-62
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ImageQuant TL 7.01 and ImageQuant TL SecurITy 8.0	1-user	28-9332-73

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