Amersham ECL Prime Western blotting detection reagent

WESTERN BLOTTING REAGENTS

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Since its introduction in 1990, the enhanced chemiluminescence (ECL) Western Blotting System portfolio has grown to accommodate applications ranging from routine protein detection to multiplex analysis using fluorescence-based Amersham™ ECL Plex™. By choosing detection systems based on ECL, researchers not only avoid the necessity of handling hazardous radioisotopes, but have a tool at their disposal that makes protein analysis faster, more sensitive, and more flexible than ever before.

The latest addition to the Amersham ECL™ family, Amersham ECL Prime, is at least twice as sensitive as Amersham ECL Plus, with a limit of detection (LOD) in the low picogram range. The reagent is characterized by greatly increased signal stability, which allows repeated exposures and makes it easier to process several blots in one experiment. In addition, the 3- to 5-fold increase in intensity of the signals emitted by Amersham ECL Prime means that proteins may be detected using primary and secondary antibodies at 3-fold higher dilutions than with Amersham ECL Plus, contributing to lower background and reducing the consumption of costly antibody reagents.

Amersham ECL Prime (Fig 1) consolidates and builds on the benefits of Amersham ECL Plus and Amersham ECL Advance™ to deliver a detection system that is sensitive, stable, precisely quantitative across a wide dynamic range of protein levels, and conservative in its the consumption of expensive antibody reagents.

Amersham ECL Prime Western blotting detection reagent deliver:

- High signal intensity and sensitivity allows the use of highly diluted primary and secondary antibodies with no reduction in sensitivity



Fig 1. Amersham ECL Prime: A high sensitivity chemiluminescent Western blotting reagent from Cytiva.

- The stable signal allows multiple exposures and makes the reagent suitable for large experimental series, allowing convenient handling time between the end of the experiment and detection
- Optimized for imaging with ImageQuant™ LAS 4000 (CCD-based imaging) and compatible with Amersham Hyperfilm™ ECL
- Compatible with Rainbow™ Molecular Weight Markers and Amersham ECL DualVue™ Western Blotting Markers
- Optimized for use with Amersham Hybond™-P (PVDF) membranes and compatible with Amersham Hybond-ECL (nitrocellulose) membranes

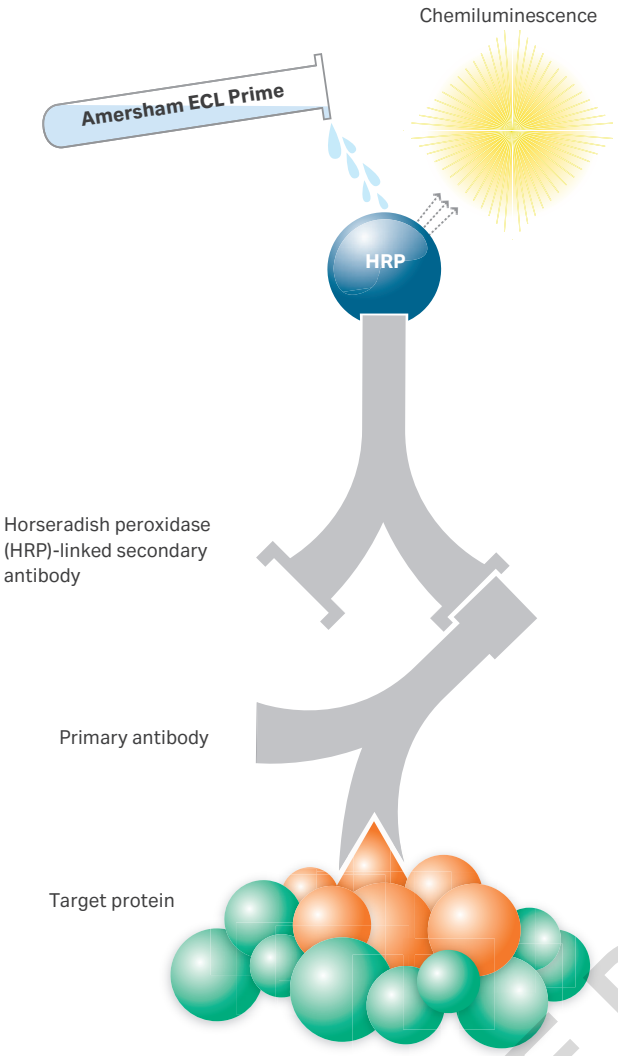


Fig 2. The reaction between Amersham ECL Prime reagent and HRP linked to a secondary antibody. HRP catalyzes the conversion of Amersham ECL Prime reagent to a sensitized molecule, which on further oxidation produces an excited product that emits light when it decays.

Chemiluminescent protein detection

Amersham ECL Prime enables the detection of minute quantities of proteins in Western blotting applications. The signal is based on light emission (chemiluminescence, see Fig 2) proportional to the amount of detected protein, and delivers precise data across a wide range of protein levels on a single blot.

Horseradish peroxidase (HRP)-catalyzed oxidation of luminol generates chemiluminescence with a wavelength of 425 nm. The improved performance of Amersham ECL Prime compared with other chemiluminescent reagents is due to the presence of an enhancer in the reagent, increasing enzyme turnover, and thereby significantly increasing both signal intensity and duration. The light signal is further intensified by the addition of a catalyst.

Amersham ECL Prime in Western blotting

Amersham ECL Prime enters Western blotting workflows at the detection stage (Fig 3), but it also has an impact on upstream procedures. The primary antibodies used as probes on the blotted membranes, for example, may be highly diluted if Amersham ECL Prime is to be used as the detection method. This is not only economically desirable, but helps avoid levels of background signals that may quench the weaker, specific interactions of interest.

The high sensitivity of Amersham ECL Prime requires that care be taken to block non-protein binding sites and that all intermediate washing steps are thoroughly carried out.

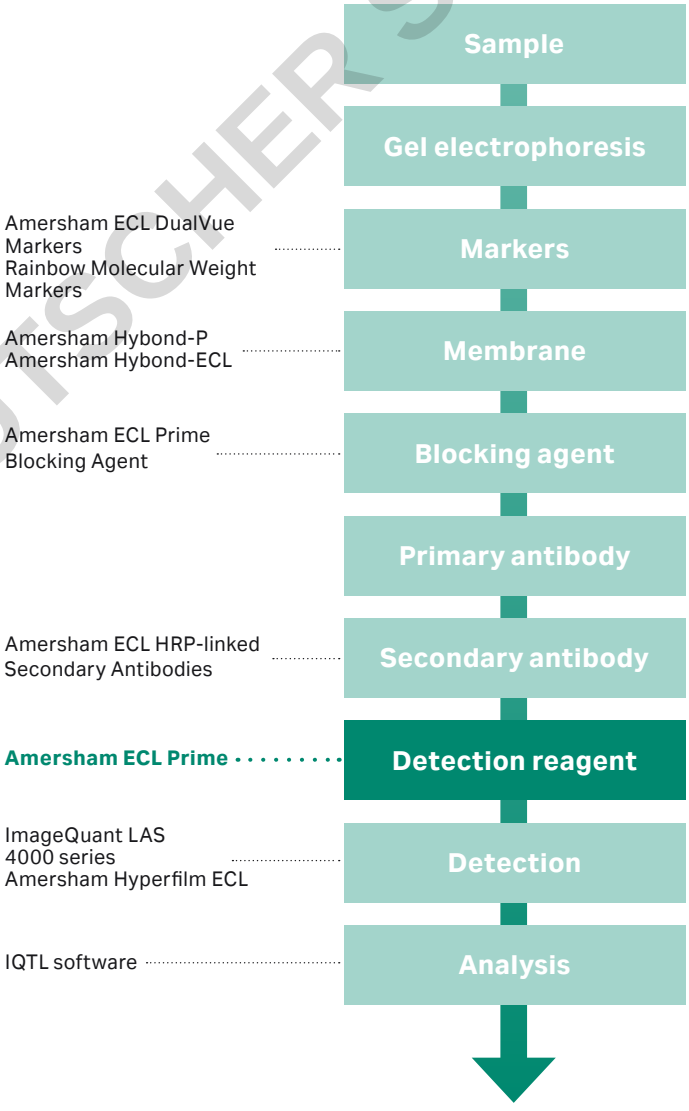


Fig 3. Amersham ECL Prime in Western blotting procedures. Most typical Western blotting experiments follow the path outlined here. The products appended to certain steps are examples from the portfolio of Cytiva, providing solutions for the entire Western blotting process, all optimized to work together.

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Amersham ECL Prime: performance characteristics

Sensitivity and precision

Amersham ECL Prime is more sensitive and leads to a linear signal response over a wider range of protein levels compared with Amersham ECL Plus. The results shown in Figure 4 were obtained after 75 sec exposure for Amersham ECL Prime, and 3 min for Amersham ECL Plus. The 4-fold increase in sensitivity (see LOD in Fig 4) and improved linear dynamic range of Amersham ECL Prime enable detection and precise quantitation of both high and low abundant proteins on the same blot after a single exposure.

Sample:	Two-fold dilution of transferrin from 2.5 ng
Membrane:	Amersham Hybond-P
Blocking:	Amersham ECL Prime Blocking Agent (Amersham ECL Prime) and 5% non-fat dry milk (Amersham ECL Plus)
Primary antibody:	Rabbit anti-transferrin (1:3000)
Secondary antibody:	HRP-conjugated anti-rabbit IgG (1:30 000)
Detection:	Amersham ECL Prime/Amersham ECL Plus
Imaging:	ImageQuant LAS 4000 mini (75 sec for Amersham ECL Prime, 3 min for Amersham ECL Plus)
LOD:	2.4 pg (Amersham ECL Prime), 9.8 pg (Amersham ECL Plus)
Dynamic range (DR):	3.0 orders of magnitude (Amersham ECL Prime), 2.4 orders of magnitude (Amersham ECL Plus)
Analysis:	ImageQuant TL 7.0 software

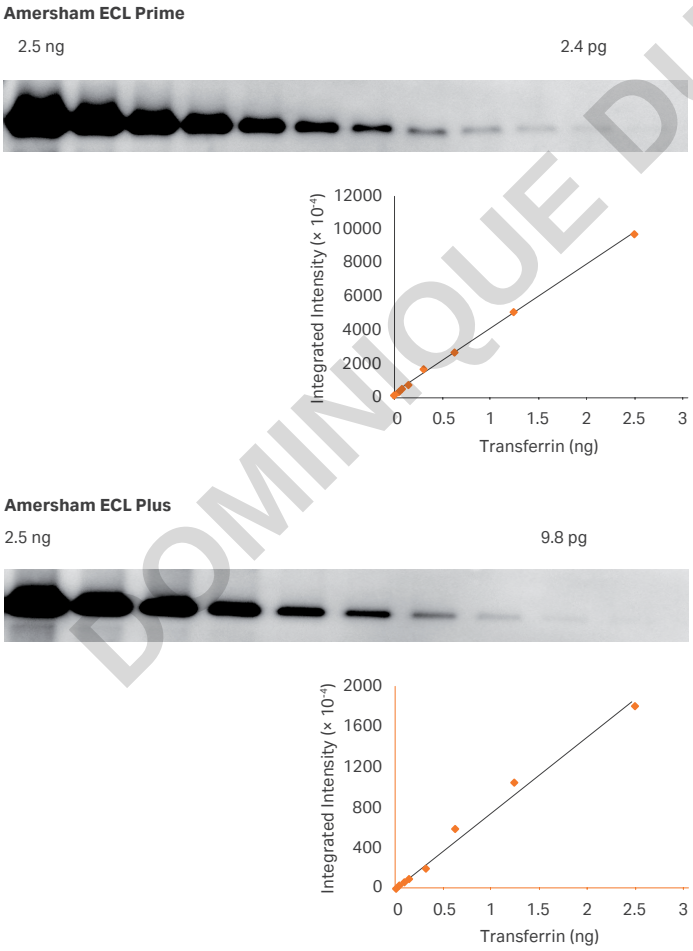


Fig 4. Western blotting detection of transferrin in a 2-fold dilution series using Amersham ECL Prime (top) and Amersham ECL Plus (bottom). The samples were blotted on to an Amersham Hybond-P (PVDF) membrane and imaged using an ImageQuant LAS 4000 mini system.

Signal stability

The chemiluminescent signal produced by Amersham ECL Prime is highly stable, with signals of sufficient intensity remaining 3 h after the addition of the reagent, even for the lowest amounts of protein tested. This enables multiple exposures and a convenient time window between the end of the experiment and analysis (Fig 5 and Table 1).

After 1 h, around 60% of the signal remained for Amersham ECL Prime whereas only 15% remained for Amersham ECL Plus.

Sample:	Two-fold dilution series of transferrin from 2.5 ng
Membrane:	Amersham Hybond-P
Blocking:	5% bovine serum albumin (BSA) in PBS-T
Primary antibody:	Rabbit anti-transferrin 1:3000
Secondary antibody:	HRP-conjugated anti-rabbit IgG (1:30 000)
Detection:	Amersham ECL Prime/Amersham ECL Plus
Imaging:	ImageQuant LAS 4000 mini (3 min for all time points)
Analysis:	ImageQuant TL 7.0 software

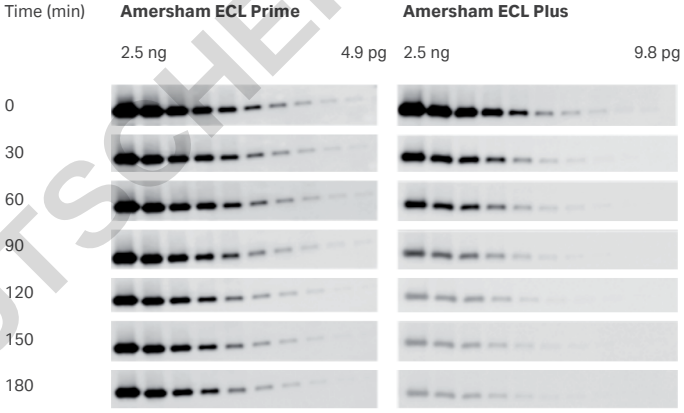


Fig 5. A side-by-side comparison of signal intensities immediately after Amersham ECL Prime reagent addition and at time points up to 3 h post reagent. A comparison is made with Amersham ECL Plus reagent over the same time course.

Table 1. Signal remaining after time points up to 3 h post reagent addition. Analysis is carried out on band 4 in each case (0.312 ng transferrin) and exposure time is 3 min for each time point.

Time point (min)	Signal intensity remaining (% of time point 0)	
	Amersham ECL Prime	Amersham ECL Plus
0	100	100
30	76	29
60	61	15
90	42	7
120	32	5
150	24	3
180	19	2

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Efficient use of antibodies

With Amersham ECL Prime, it is possible to detect lower quantities of protein than with Amersham ECL Plus, even if primary antibodies are used at a much higher dilution (Fig 6). Signal intensities and LOD are similar when antibodies are used at 1:10 000 (primary) and 1:50 000 (secondary) with Amersham ECL Prime compared with dilutions of 1:3000 and 1:30 000 with Amersham ECL Plus.

Sample:	Two-fold dilution series of NIH 3T3 cell lysates from 10 µg total protein
Membrane:	Amersham Hybond-P
Blocking:	5% BSA in PBS-T
Primary antibody:	Rabbit anti-β-catenin (1:3000 to 1:10 000)
Secondary antibody:	HRP-conjugated anti-rabbit IgG (1:30 000 to 1:50 000)
Detection:	Amersham ECL Prime/ Amersham ECL Plus
Imaging:	ImageQuant LAS 4000 mini (5 min, primary antibodies at 1:3000 or 1:5000; 7 min, primary antibodies 1:7000 or 1:10 000)
Analysis:	ImageQuant TL 7.0 software

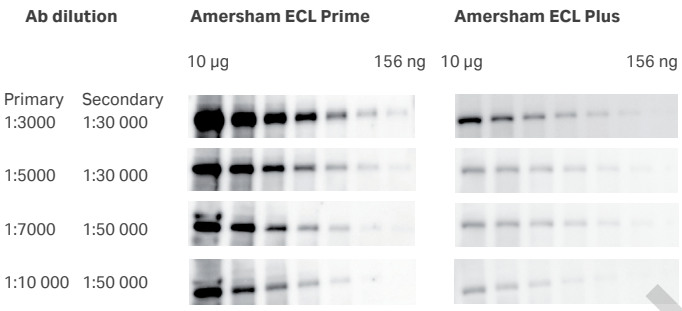


Fig 6. Comparison of Amersham ECL Prime and Amersham ECL Plus Western blotting detection of β-catenin in NIH 3T3 whole cell lysates in a 2-fold dilution series using different dilutions of rabbit anti-β-catenin and HRP-conjugated anti-rabbit IgG.

Product compatibility

Amersham ECL Prime has been developed for optimal performance with specific products from the Western blotting portfolio from Cytiva, for example Amersham Hybond-P membranes. It is compatible, however, with other products commonly used in Western blotting protocols. For example, excellent results can also be obtained using nitrocellulose Amersham Hybond-ECL membranes, as well as molecular weight markers or blocking reagents not specifically recommended for use with Amersham ECL Prime.

Relative quantitation of different levels of target protein in cell lysates

The high sensitivity of Amersham ECL Prime combined with the broad dynamic range of CCD-based imagers, such as ImageQuant LAS 4000 mini, enables relative quantitation of target proteins with confidence, by normalization with levels of an unregulated housekeeping protein on the same blot (Fig 7). By monitoring expression levels of housekeeping proteins, it is possible to ensure that a similar amount of total cell lysate is added to each lane.

Sample:	10 µg total protein from NIH 3T3 cell lysates (IFNα-treated/untreated cells in different ratios) or HeLa cell lysates (anisomycin-treated/untreated cells in different ratios)
Membrane:	Amersham Hybond-P
Blocking:	5% BSA in PBS-T
Primary antibody:	Rabbit anti-β-catenin (1:3000), mouse anti-pSTAT3 (1:3000) or mouse anti-actin (1:3000)
Secondary antibody:	HRP-conjugated anti-rabbit IgG (1: 30 000) or HRP-conjugated anti-mouse IgG (1:30 000)
Detection:	Amersham ECL Prime
Imaging:	ImageQuant LAS 4000 mini (3 min, β-catenin; 1 min, actin)
Analysis:	ImageQuant TL 7.0 software

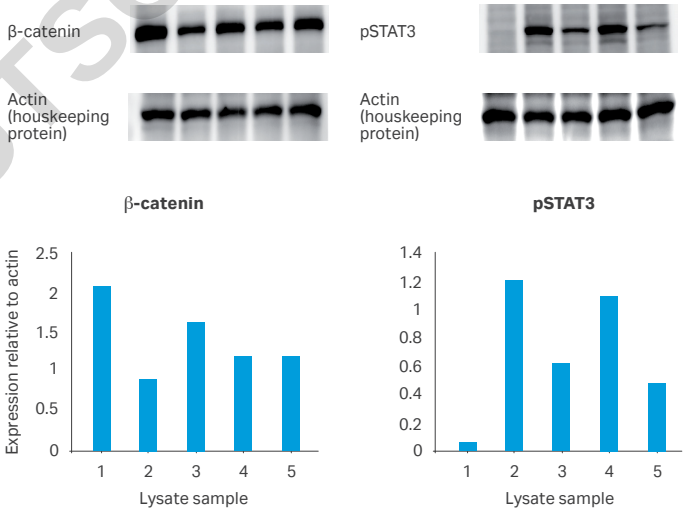


Fig 7. Western blotting detection of (A) β-catenin in 5 different NIH 3T3 cell lysates and (B) Tyr^{705}-phosphorylated STAT3 (pSTAT3) in 5 different HeLa cell lysates. In each case, the target proteins were relatively quantitated by normalization with actin levels in the same lysate, on a single blot. Note that although levels of β-catenin appear similar on a visual examination of the blot, analysis using Amersham ECL Prime and ImageQuant LAS 4000 mini shows that the protein levels are clearly different.

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Detection of low abundance phosphorylated proteins

STAT3 is a transcription activator involved in melanoma progression and host immunity. The protein is activated by phosphorylation of a specific tyrosine residue, upon treatment of cells with certain cytokines, including IFNα.

A 2-fold dilution series of lysates from IFNα-treated HeLa cells, starting at 12.5 µg total protein, was run on a polyacrylamide gel and blotted onto an Amersham Hybond-P membrane. Phosphorylated STAT3 was then detected using a specific antibody followed by Amersham ECL Prime (Fig 8).

Sample:	Two-fold dilution series of IFNα-treated HeLa cell lysates from 12.5 µg total protein
Membrane:	Amersham Hybond-P
Blocking:	5% BSA in PBS-T
Primary antibody:	Mouse monoclonal anti-pSTAT3 (Tyr^{705}) (1:3000)
Secondary antibody:	HRP-conjugated anti-mouse IgG (1:30 000)
Detection:	Amersham ECL Prime/ Amersham ECL Plus
Imaging:	ImageQuant LAS 4000 mini (5 min)
Analysis:	ImageQuant TL 7.0 software

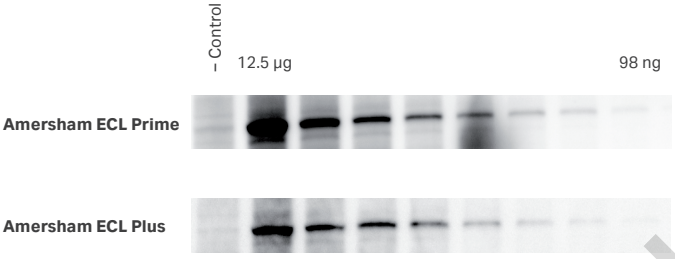


Fig 8. Amersham ECL Prime and Amersham ECL Plus Western blotting detection of pSTAT3 in IFNα-treated HeLa cells. The more intense signals emitted by Amersham ECL Prime led to improved quality of protein detection in terms of sensitivity when compared with Amersham ECL Plus. The control lane contains 6.25 µg total protein from untreated cells.

Ordering Information

Product	Code number
Amersham ECL Prime Western Blotting Detection Reagent for 1000 cm^2 membrane*	RPN2232
Amersham ECL Prime Western Blotting Detection Reagent for 3000 cm^2 membrane†	RPN2236

* Includes Solution A (luminol solution, 50 ml) and Solution B (peroxide solution, 50 ml), sufficient for 1000 cm^2 membrane.

† Includes Solution A (luminol solution, 3 × 50 ml) and Solution B (peroxide solution, 3x 50 ml), sufficient for 3000 cm^2 membrane.

Related products‡

Amersham Hybond-P (20 × 20 cm), 10 sheets	RPN2020F
Amersham ECL Prime blocking reagent, 40 g	RPN418
Amersham ECL Mouse IgG, HRP-Linked Whole Ab (from sheep), 1 ml	NA931-1ML
Amersham ECL Rabbit IgG, HRP-Linked Whole Ab (from donkey), 1 ml	NA934-1ML
Amersham Full-Range Rainbow Molecular Weight Markers 250 µl	RPN800E
Amersham ECL DualVue Western Blotting Markers (25 loadings)	RPN810

‡ For a more comprehensive list of related products, please refer to www.cytiva.com/ecl

Imaging systems

ImageQuant LAS 500	29-0050-63
ImageQuant LAS 4000	28-9558-10

Software and accessories

ImageQuant TL 7.0 and IQTL SecurITy 8.0 Software packages (with Getting Started guide)	28-9380-94
ImageQuant TL 7.01 and ImageQuant TL SecurITy 8.0 (single user)	28-9332-73

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ECL Advance contains Lumigen TMA-6 substrate and is sold under exclusive license from Lumigen Inc.

ECL Plus contains Lumigen PS3 substrate and is sold under exclusive license from Lumigen Inc.

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