Increased sensitivity and stability in Western blotting using Amersham™ ECL™ Prime

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Amersham ECL Prime provides a Western blotting detection system that is sensitive, stable, precisely quantitative across a wide dynamic range of protein levels, and conservative in consumption of expensive antibody reagents. This article describes the improvements made to the new Amersham ECL Western blotting detection system compared to its predecessor, Amersham ECL Plus.

Introduction

Since the introduction in 1990 of the first enhanced chemiluminescence (ECL) reagent for Western blotting, Amersham ECL, the portfolio of products has grown to cover all Western blotting requirements from confirmatory detection to multiplexed fluorescent detection. Today, ECL based detection systems offer a tool for highly sensitive, stable, and flexible Western blotting analysis enabling precise detection and quantitation of protein levels across a wide range.

The latest improvement to the portfolio, Amersham ECL Prime, enables detection of minute quantities of proteins in Western blotting applications. The signal is based on chemiluminescence (light emission) 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) catalyses oxidation of the luminol reagent of ECL Prime, generating chemiluminescence with an emission 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, thereby significantly increasing both signal intensity and duration. The light signal is further intensified by the addition of a catalyst. 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. The three- to five-fold increase in intensity of the signals emitted by Amersham ECL Prime compared with ECL Plus means that proteins can be detected using primary and secondary antibodies at three-fold higher dilutions than with Amersham ECL Plus, contributing to lower background and reduced consumption of costly antibody reagents.

The reagent is simple to adapt to established laboratory protocols due to compatibility with most commercially available Western blotting reagents, molecular weight markers, membranes, and blocking reagents.

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 1 were obtained after 75 s exposure for Amersham ECL Prime and 3 min for Amersham ECL Plus. The four-fold increase in sensitivity (limit of detection [LOD]) was 2.4 pg for Amersham ECL Prime and 9.8 pg for Amersham ECL Plus.

Signal stability

The chemiluminescent signal produced by Amersham ECL Prime is highly stable with a sustained detection limit at 180 min after addition of Amersham ECL Prime, using constant exposure time. At 1 h after addition of the reagent, around 60% of the signal remained for Amersham ECL Prime while only 15% remained for Amersham ECL Plus.

Sample:	Two-fold dilution series 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 s 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

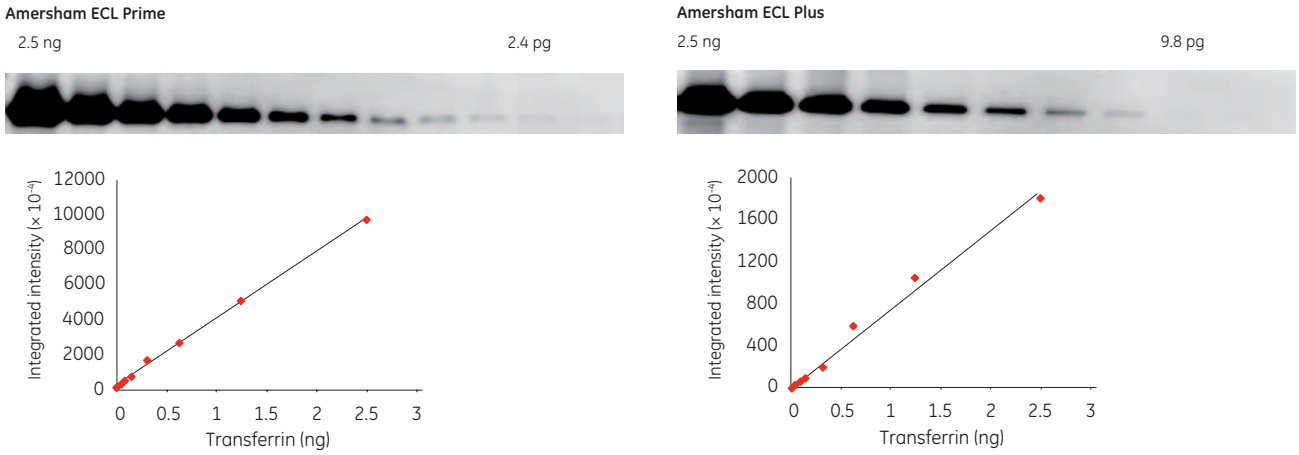

Fig 1. Western blotting detection of transferrin in a two-fold dilution series using Amersham ECL Prime (left) and Amersham ECL Plus (right). The samples were blotted on to an Amersham Hybond-P (PVDF) membrane and imaged using an ImageQuant LAS 4000 mini system.

Figure 2 and Table 1 show how Amersham ECL Prime enables multiple exposures and a convenient time window between the end of the experiment and analysis, even for the lowest amounts of protein tested.

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 exposure)
Analysis:	ImageQuant TL 7.0 software

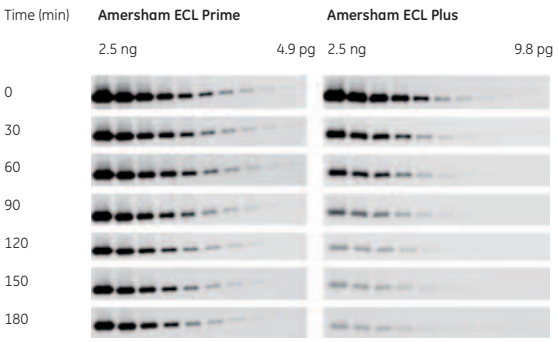

Fig 2. A side-by-side comparison of signal intensities immediately after addition of Amersham ECL Prime reagent and up to 180 min after addition of the reagent. A comparison is made with Amersham ECL Plus reagent over the same time course. Analysis was performed on band 4 in each case (0.312 ng transferrin) and exposure time was 3 min for each time point.

Table 1. Signal remaining from 0 to 180 min after addition of Amersham ECL Prime

Time point (min)	Signal intensity remaining (% relative to 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

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 3). 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.

Although levels of β-catenin appear similar on a visual examination of the Western blot shown in Figure 3, analysis using Amersham ECL Prime and ImageQuant LAS 4000 mini showed that the protein levels were clearly different.

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 exposure, β-catenin; 1 min exposure, actin)
Analysis:	ImageQuant TL 7.0 software

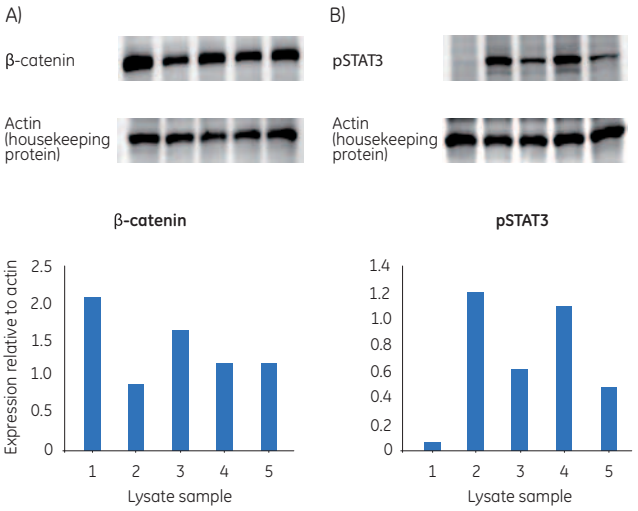

Fig 3. Western blotting detection of **A)** β-catenin in five different NIH 3T3 cell lysates and **B)** Tyr705-phosphorylated STAT3 (pSTAT3) in five different HeLa cell lysates. In each case, the target proteins were relatively quantitated by normalization with actin levels in the same lysate on a single blot (lower panels).

Conclusion

Amersham ECL Prime is at least twice as sensitive as Amersham ECL Plus, with an 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.

Reference

1. Kricka, L. J. *et al.* Chemiluminescent methods for detecting and quantitating enzyme activity. *Methods Enzymol.* **305**, 370–390 (2000).

Ordering information

Product	Code number
Amersham ECL Prime Western Blotting Detection Reagents for 1000 cm² membrane	RPN2232

For more information on products for Western blotting detection reagents from GE Healthcare, visit www.gelifesciences.com/eclprime