Amersham ECL start Western blotting detection reagent

PROTEIN ANALYSIS

Amersham™ ECL™ start Western blotting detection reagent (Fig 1) is a luminol-based chemiluminescent substrate designed for the detection of proteins immobilized to a membrane. It is a chemiluminescent detection reagent in the Amersham ECL product family that is recommended for confirmatory analysis and detection of high to medium levels of endogenous proteins. Amersham ECL start has a long signal duration, which enables multiple exposures of the membrane, plus a convenient time window between experiment and analysis.

Amersham ECL start Western blotting detection reagent delivers:

- **Flexibility:** Long signal duration provides assay design flexibility and robust detection of large experimental sets
- **Low background:** Produces low background, regardless of the method of detection
- **Stability of working solution:** The working solution is stable for 5 d
- **Long shelf life:** A long shelf life of 18 mo is convenient for those who run Western blots infrequently

The signal from Amersham ECL start Western blotting detection reagent is based on the emission of light from Horseradish peroxidase (HRP)-catalyzed oxidation of luminol, which generates chemiluminescence with a wavelength of 428 nm. The light signal is easily detected with X-ray film such as Amersham Hyperfilm or a CCD imager like Amersham Imager 600.

Fig 1. Amersham ECL start Western blotting detection reagent delivers a chemiluminescent signal that allows you to detect high to medium abundance proteins.

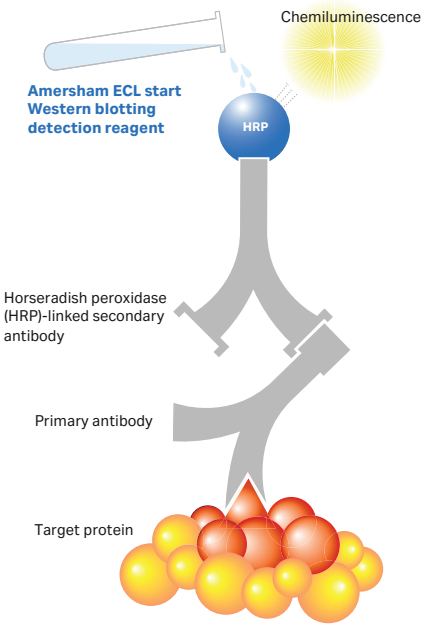


Fig 2. The reaction between Amersham ECL start Western blotting detection reagent and HRP linked to a secondary antibody. The HRP-conjugated secondary antibody binds to the primary antibody that is specifically bound to the target protein on the membrane. After the addition of a luminol peroxide detection reagent, the HRP enzyme catalyzes the oxidation of luminol in a multistep reaction. The reaction is accompanied by the emission of low intensity light at 428 nm.

Application of Amersham ECL start in Western blotting

Amersham ECL start Western blotting detection reagent is typically used at the detection stage of the Western blotting workflow (Fig 4). Amersham ECL start has a dynamic range of 2.1, which makes it an optimal reagent for confirmatory Western blotting. The reagent can also be used in quantitative applications, but only in those cases where you expect small variations in protein expression.

In a 2-fold dilution series with NIH/3T3 cell lysate starting at 25 µg, GAPDH was detected up to 1.56 µg. The light output and linear range of detection indicate that Amersham ECL start Western blotting detection reagent is an optimum reagent for high to medium protein levels.

Sample:	NIH/3T3 cell lysate in a 2-fold dilution range from 25 µg total protein
Membrane:	Amersham Protran Premium 0.45 NC
Blocking:	Skimmed milk powder (5% in PBS-T)
Primary Ab:	Rabbit anti GAPDH diluted 1:5000
Secondary Ab:	HRP-conjugated anti-rabbit IgG diluted 1:50 000
Imaging:	Amersham Hyperfilm ECL
Exposure time:	5 min

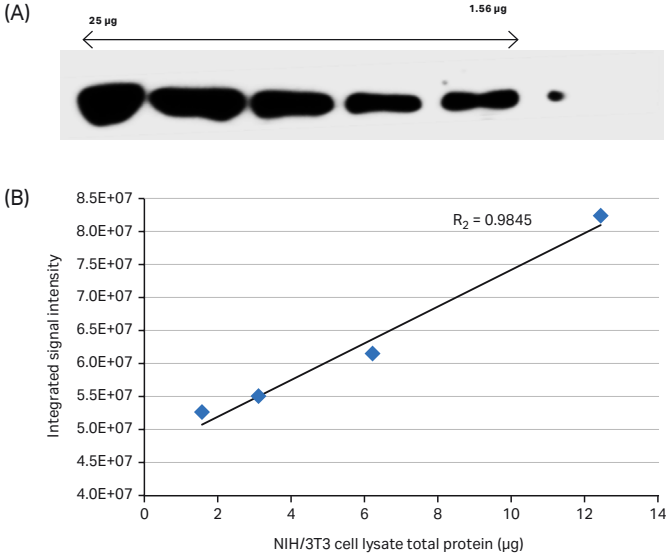


Fig 3. Western blotting detection of GAPDH protein in NIH/3T3 cell lysate using Amersham ECL start Western blotting detection reagent shows its limit of detection (5 bands) and high signal intensity. The resulting blot is shown in (A), and the linearity of signal intensity from 12.5 µg total protein in (B).

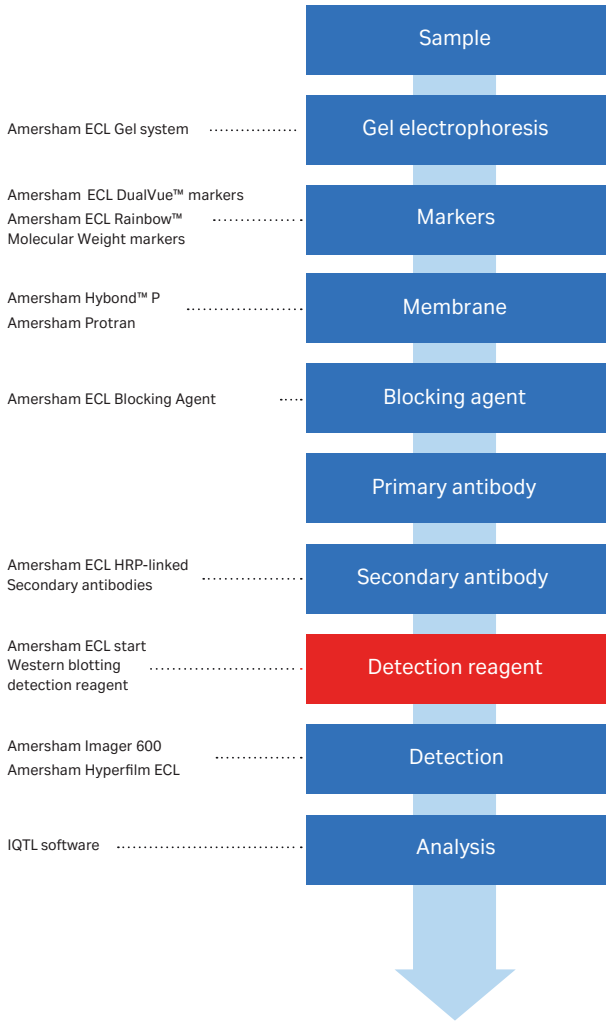


Fig 4. A schematic representation of the role of Amersham ECL start Western blotting detection reagent in a typical Western blotting procedure. We supply products spanning the entire spectrum of the Western blotting process.

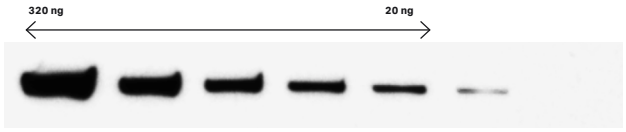
Compatibility with X-ray film and CCD imaging

Sensitivity and precision

In a 2-fold dilution series of recombinant β-galactosidase protein starting at 320 ng, 20 ng of protein was repeatedly detected at the lower end (Fig 5A). The low background produces high signal-to-noise, which clearly demonstrates the high compatibility between Amersham ECL start and X-ray film. In a 2-fold dilution series of NIH/3T3 cell lysate starting at 10 µg of total protein, ERK 1/2 was detected at levels as low as 0.31 µg (Fig 5B). The signal response was linear. The signal was easily detected with a CCD imager. In addition, the overlay functionality allows fast verification of the molecular weight of the protein.

Sample:	β-galactosidase protein in a 2-fold dilution series starting at 320 ng protein
Membrane:	Amersham Protran Premium 0.45 NC
Blocking:	Skimmed milk powder (5% in PBS-T)
Primary Ab:	Rabbit anti-β-galactosidase diluted 1:5000
Secondary Ab:	HRP-conjugated anti-rabbit IgG diluted 1:50 000
Imaging:	X-ray film
Exposure time:	5 min
Limit of detection:	20 ng (repeatedly)

(A)



Sample:	NIH/3T3 cell lysate in a 2-fold dilution range from 10 µg total protein
Membrane:	Amersham Hybond P 0.45 PVDF
Blocking:	Amersham ECL Prime blocking agent (2% in PBS-T)
Primary Ab:	Rabbit anti-ERK 1/2 diluted 1:5000
Secondary Ab:	HRP-conjugated anti-rabbit IgG diluted 1:50 000
Imaging:	Amersham Imager 600
Exposure time:	5 min

(B)

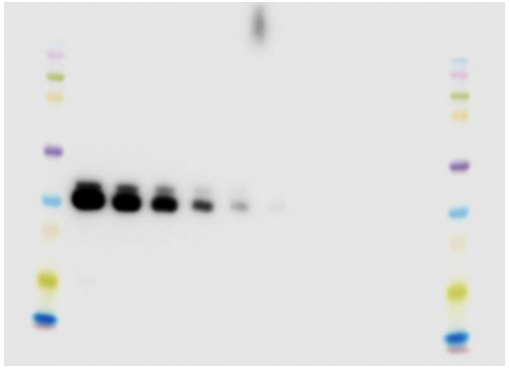


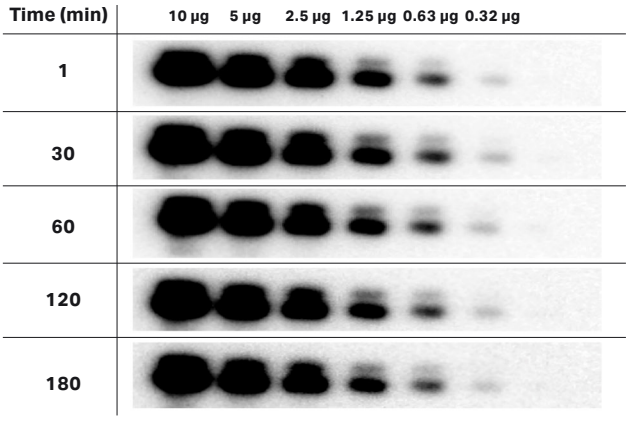
Fig 5. Western blotting detection using Amersham ECL start, showing resulting blots from β-galactosidase protein (A) and ERK 1/2 in NIH/3T3 lysate in (B). The data demonstrates compatibiltiy between Amersham ECL start and X-ray film. The high signal intensity fully utilizes the advantages of CCD camera-based imaging equipment like Amersham Imager 600.

Signal duration

We assessed the signal duration of Amersham ECL start for Western blotting detection of ERK 1/2 in NIH/3T3 cell lysate. We detected the first emitted signal immediately (i.e., 1 min) after the working solution was removed from the membrane, and the signal intensity was followed at time points of up to 180 min post reagent addition. Amersham ECL start Western blotting detection reagent retained sufficient signal 3 h after addition of the working solution. This gives you a convenient time window between the end of an experiment and the beginning of analysis thus allowing for multiple exposures.

Sample:	NIH/3T3 cell lysate in a 2-fold dilution range from 10 µg total protein
Membrane:	Amersham Hybond P 0.45 PVDF
Blocking:	Amersham ECL Prime blocking agent (2% in PBS-T)
Primary Ab:	Rabbit anti-ERK 1/2 diluted 1:5000
Secondary Ab:	HRP-conjugated anti-rabbit IgG diluted 1:50 000
Imaging:	Amersham Imager 600
Exposure time:	5 min

(A)



(B)

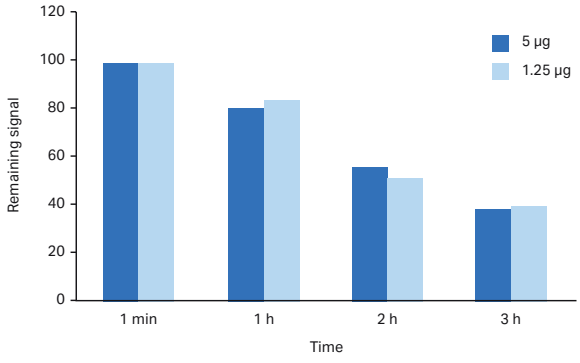


Fig 6. An assessment of signal duration for Amersham ECL start Western blotting detection reagent over time indicated that; (A). The signal duration allows for the sustained detection of high to medium protein levels for up to 3 h; (B) The decline over time in signal intensity is proportional to the protein amount (remaining signal intensity for 5 µg and 1.25 µg bands are shown at 1 min to 3 h).

Working solution can be used for up to 5 d

We used the working solution of Amersham ECL start on days 0 and 5 to detect purified β-gal protein with Amersham ECL start (Fig 7A). After 5 d of storage at 4°C to 8°C, the remaining signal was strong enough to be used to confirm the presence of protein. When we evaluated the signal intensity of the protein bands at 160 and 40 ng, respectively; the results showed that the protein signal declined proportionally to protein amount (Fig 7B).

Sample:	β-gal protein in a 2-fold dilution series starting at 320 ng protein
Membrane:	Amersham Protran Premium 0.45 NC
Blocking:	Skimmed milk powder (5% in PBS-T)
Primary Ab:	Rabbit anti-β-gal diluted 1:5000
Secondary Ab:	HRP-conjugated anti-rabbit IgG diluted 1:50 000
Imaging:	X-ray film
Exposure time:	5 min

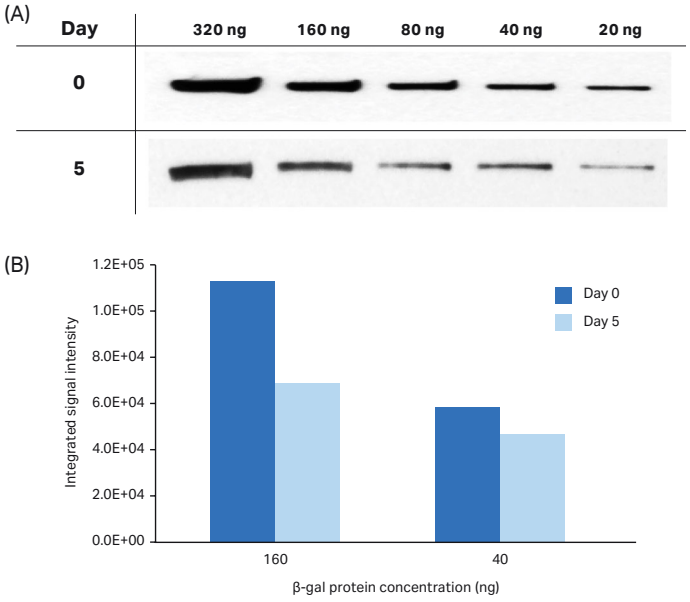


Fig 7. Detection of high to medium β-gal protein is; (A) Sustained by using a working solution that was prepared 5 d earlier; and (B) Shows a comparison of the signal intensities for 2 bands.

Ordering information

Product†	Code number
Amersham ECL start Western Blotting Detection Reagent for 2000 cm^2 membrane	RPN3243
Amersham ECL start Western Blotting Detection Reagent for 4000 cm^2 membrane	RPN3244

† These products are available from Cytiva distributors www.cytiva.com/distributors

*Related products**

Amersham Protran 0.2 NC 1 roll 30 cm × 4 m	10600001
Amersham Hybond P 0.2 PVDF 1 roll 26 cm × 4 m	10600021
Amersham ECL Prime Blocking Agent, 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 ECL Rainbow Markers 250 µl	RPN800E
Amersham ECL DualVue Western Blotting Markers (25 loadings)	RPN810
Amersham ECL Gel Box	28-9906-08
Amersham ECL Gel, 10% (pack of 10 gels)	28-9898-04
Amersham ECL Gel, 4-20% (pack of 10 gels)	28-9901-54

Imaging systems and X-ray film

Amersham Imager 600	29-0834-61
ImageQuant™ LAS 500	29-0050-63
Amersham Hyperfilm ECL, 18 × 24 cm (50 sheets)	28-9068-36

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

cytiva.com/amershamwb

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