Amersham ECL Select

WESTERN BLOTTING REAGENTS

Amersham™ ECL Select™ (Fig 1) is a highly sensitive reagent for chemiluminescent Western blotting detection. It is the most sensitive chemiluminescent detection reagent in the Amersham ECL™ product family. The dynamic range of Amersham ECL Select is complementary to that of Amersham ECL and Amersham ECL Prime. The outstanding signal intensity of Amersham ECL Select makes it suitable for the most demanding Western blotting applications including the detection of minute protein quantities. You can perform multiple exposures because of the long lasting signals of Amersham ECL Select and the convenient time window between experiment and analysis.

Fig 1. Amersham ECL Select delivers an intense chemiluminescent signal that allows you to detect low abundant proteins.

Amersham ECL Select delivers:

Improved sensitivity: Exceptional signal intensity and sensitivity allow you to detect very low abundant proteins.

Quantitative measurements: Wide linear dynamic range enables quantitation of medium to very low protein levels.

Flexibility: Optimized for imaging with ImageQuant™ LAS systems (CCD-based imaging), and it is also compatible with Amersham Hyperfilm™ product range.

Versatility: Works with a wide range of antibody dilutions, which allows you to use highly diluted antibodies and reduces the need for lengthy optimization.

The signal from Amersham ECL Select is based on the emission of light from Horseradish peroxidase (HRP)-catalyzed oxidation of luminol, which generates chemiluminescence with a wavelength of 425 nm. Since the light output is proportional to the amount of protein detected, this results in a precise quantitation across a wide range of protein levels on a single blot.

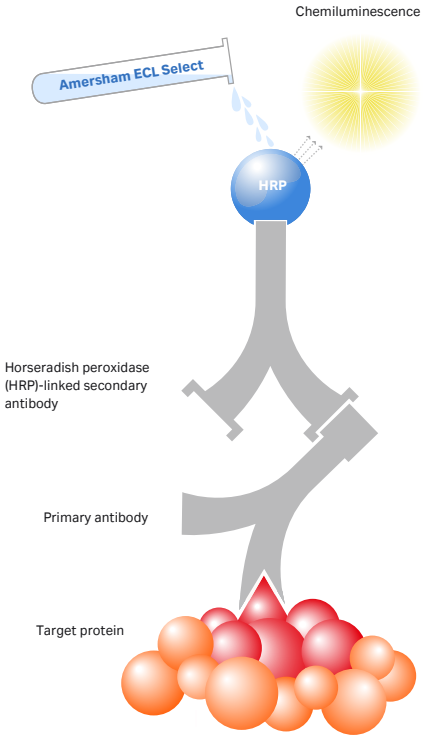

Fig 2. The reaction between Amersham ECL Select reagent and HRP linked to a secondary antibody. HRP catalyzes the conversion of Amersham ECL Select reagent to a sensitized molecule, which on further oxidation produces an excited product that emits light when it decays.

Application of Amersham ECL Select in Western blotting

Amersham ECL Select is typically used at the detection stage of the Western blotting workflow (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 you use Amersham ECL Select for detection. This reduces cost and avoids levels of background signals that may quench the weaker, specific interactions of interest.

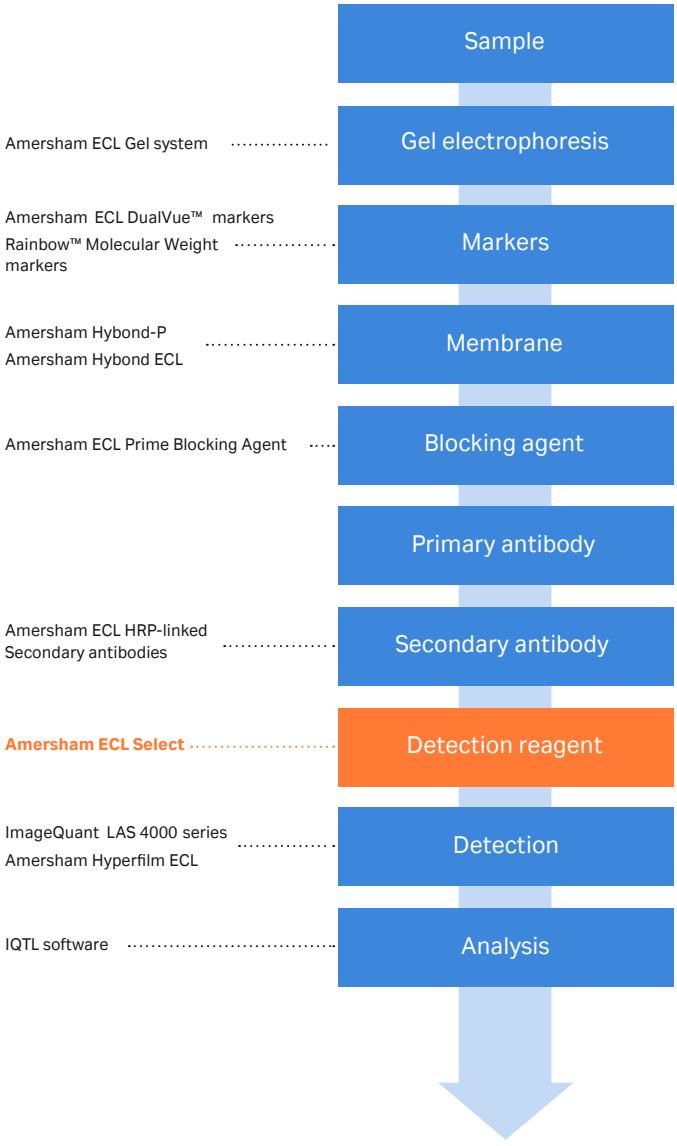

Fig 3. A schematic representation of the role of Amersham ECL Select in a typical Western blotting procedure. As exemplified, we supply products spanning the entire spectrum of the Western blotting process.

Performance characteristics of Amersham ECL Select

Sensitivity and precision

In a 2-fold dilution series of HeLa cell lysate starting at 5 µg of total protein, ERK ½ was detected at levels as low as 5 ng (Fig 4A and B). The signal response was linear over a wide range of protein concentrations. The high sensitivity and signal intensity together with the broad linear dynamic range makes Amersham ECL Select suitable for qualitative as well as quantitative analysis. Amersham ECL Select produces very high light output and this allows you to use highly diluted antibodies in your experiments.

Sample:	HeLa cell lysate in a 2-fold dilution series starting at 5 µg total protein
Membrane:	Amersham Hybond™-P
Blocking:	Amersham ECL Prime blocking agent (2% in PBS-T)
Primary Ab:	Rabbit anti ERK ½ diluted 1:10 000
Secondary Ab:	HRP conjugated anti-rabbit IgG diluted 1:100 000
Imaging:	ImageQuant LAS 4000 mini
Exposure time:	1 min
Limit of detection:	5 ng (total protein)
Linear dynamic range:	2.7 orders of magnitude

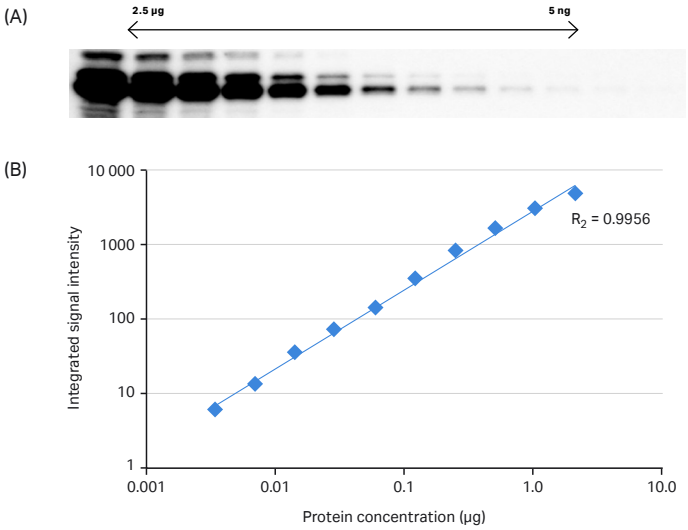

Fig 4. High sensitivity Western blotting detection of ERK ½ in HeLa cell lysate using Amersham ECL Select. Resulting blot shown in (A) and integrated signal intensity in; (B). The data shows that Amersham ECL Select delivers high sensitivity, and that the signal response is linear over a wide range of protein levels.

We compared the sensitivity and signal intensity of Amersham ECL Select to that of Amersham ECL Advance™ in a Western blotting experiment to detect ERK ½. Both reagents showed very high sensitivity and produced equal detection limits for ERK ½ (5 ng total protein) (Fig 5A and B). However, Amersham ECL Select produced 3-fold higher signal intensity (Fig 5C) with a sustained low background, resulting in brighter and clearer protein bands. We used optimized antibody dilutions for each detection reagent and each blot was exposed for 1 min using ImageQuant LAS 4000 imaging system.

Sample:	HeLa cell lysate in a 2-fold dilution series starting at 5 µg total protein
Membrane:	Amersham Hybond-P
Blocking:	Amersham ECL Prime blocking agent (2% in PBS-T)
Primary Ab:	Rabbit anti ERK ½ diluted 1:10 000 (Amersham ECL Select), and 1:30 000 (Amersham ECL Advance)
Secondary Ab:	HRP conjugated anti-rabbit IgG diluted 1:100 000 (Amersham ECL Select), and 1:300 000 (Amersham ECL Advance)
Imaging:	ImageQuant LAS 4000
Exposure time:	1 min

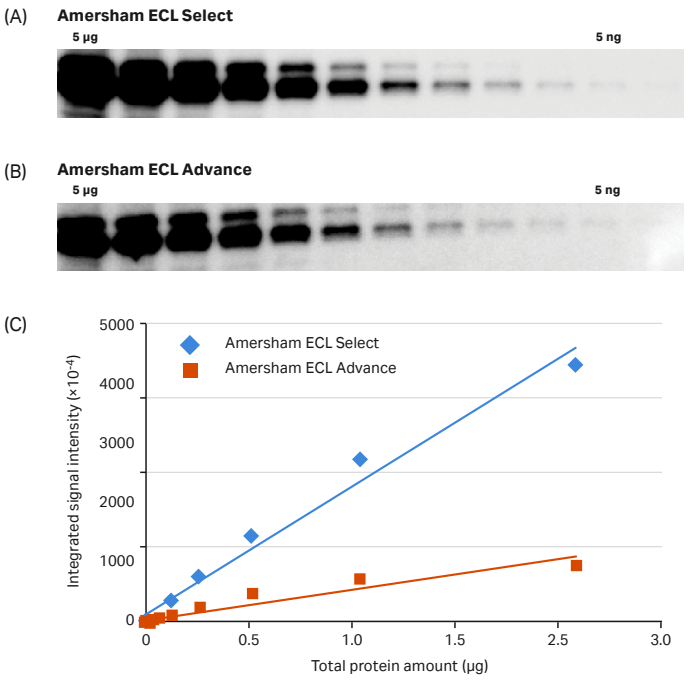

Fig 5. Comparative performance evaluation of Amersham ECL Select and Amersham ECL Advance. ECL Select shows similar limit of detection (5A and B), with significantly brighter bands due to the higher signal intensity, illustrated in 5C.

Signal intensity and duration

We compared the signal intensity and duration of Amersham ECL Select to that of Amersham ECL Advance for Western blotting detection of ERK ½ in HeLa cell lysate. We detected the emitted signals immediately after addition of the respective detection reagent, and followed the signal intensity at time points up to two hours after reagent addition. Blots for Amersham ECL Select and Amersham ECL Advance were imaged side by side, using same exposure times. Amersham ECL Select gives a significantly higher signal output, with sufficient signals remaining 2 h after addition of the reagent, even for the lowest amount of protein tested. This gives you a convenient time window between the end of an experiment and the beginning of analysis thus allowing for multiple exposures.

Sample:	HeLa cell lysate in a 2-fold dilution series starting at 5 µg total protein
Membrane:	Amersham Hybond-P
Blocking:	Amersham ECL Prime blocking agent (2% in PBS-T)
Primary Ab:	Rabbit anti ERK ½ diluted 1:10 000 (Amersham ECL Select), and 1:30 000 (Amersham ECL Advance)
Secondary Ab:	HRP conjugated anti-rabbit IgG diluted 1:100 000 (Amersham ECL Select), and 1:300 000 (Amersham ECL Advance)
Imaging:	ImageQuant LAS 4000
Exposure time:	3 min

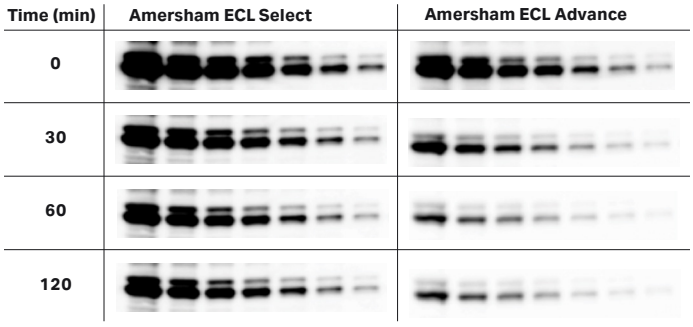

Fig 6. A side-by-side comparison of signal durations for Amersham ECL Select and Amersham ECL Advance. The high signal intensity of Amersham ECL Select allows for sustained detection of very low protein levels for up to 2 h.

Efficient use of antibodies

Amersham ECL Select allows you to perform high sensitivity Western blots with highly diluted antibodies. We performed a Western blotting detection of PP2A in a 2-fold-dilution series of NIH/T3T cell lysate to demonstrate that minute levels of protein can be detected using a wide range of primary antibody dilutions (Fig 7). The tolerance for different antibody dilutions allows you to save your precious antibodies, and in addition, it reduces the need for lengthy optimization.

Sample:	NIH/T3T cell lysate
Membrane:	Amersham Hybond-P
Blocking:	Amersham ECL Prime blocking agent (2% in PBS-T)
Primary Ab:	Rabbit anti-PP2A
Secondary Ab:	HRP conjugated anti-rabbit IgG 1:100 000
Imaging:	ImageQuant LAS 4000
Exposure time:	3 min

Primary Ab dilution	Secondary Ab dilution	10.0	5.0	2.5	1.2	0.6	0.3 µg
1:3000	1:100 000						
1:5000	1:100 000						
1:10 000	1:100 000						
1:15 000	1:100 000						

Fig 7. Amersham ECL Select provides high sensitivity using a wide dilution range of antibodies.

CCD-based imaging or X-ray film

The chemiluminescent signals of Amersham ECL Select can be captured by using either an CCD-based imager such as ImageQuant LAS series, or X-ray film such as Amersham Hyperfilm product range. To demonstrate this we performed Western blotting detection of endogenous p38 in NIH/T3T cell lysates loaded at 30 µg (lane 1) and 15 µg (lane 2) total protein amount. The same membrane was detected using both ImageQuant LAS 4000 mini and Amersham Hyperfilm ECL, and the results (Fig 8) depict equal performance with bright protein bands and low background.

Sample:	NIH/T3T cell lysate, 30 µg (1) and 15 µg (2) total protein
Membrane:	Amersham Hybond-P
Blocking:	Amersham ECL Prime blocking agent (2% in TBS-T)
Primary Ab:	Mouse monoclonal anti-p38 diluted 1:5000
Secondary Ab:	HRP conjugated anti-mouse IgG diluted 1:100 000
Imaging:	ImageQuant LAS 4000 mini and Amersham Hyperfilm ECL
Exposure time:	1 min

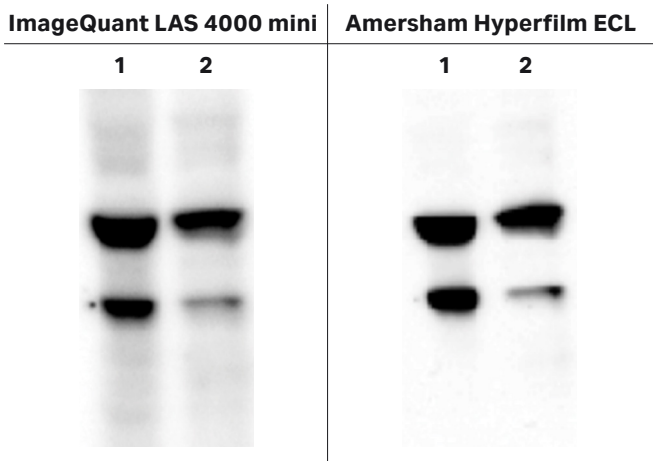

Fig 8. The high signal intensity and low background you obtain from using Amersham ECL Select allows you to use either and X-ray film or CCD-based imager for imaging.

Detection of low abundant endogenous proteins

To show that Amersham ECL Select enables detection of low abundant endogenous proteins, we performed Western blotting detection of pStat3 in cell lysate from INF-α-treated HeLa cells. The high sensitivity of Amersham ECL Select allowed for detection of pStat3 in samples with minute protein concentration (Fig 9).

Sample:	Cell lysate from INF-α-treated HeLa cells in a 2-fold dilution series starting at 5 µg total protein
Membrane:	Amersham Hybond-P
Blocking:	Amersham ECL Prime blocking agent (2% in TBS-T)
Primary Ab:	Mouse monoclonal anti-pStat3 diluted 1:5000
Secondary Ab:	HRP conjugated anti-mouse IgG diluted 1:100 000
Imaging:	ImageQuant LAS 4000 mini
Exposure time:	2 min

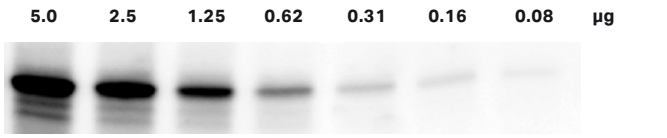

Fig 9. Detection of minute levels of pStat3 in INF-α-treated HeLa cells.

In a similar experiment, endogenous TAB 1 in 293 T cell lysate could be successfully detected at low levels (Fig 10).

Sample:	293 T cell lysate in a 2-fold dilution series starting at 2 µg total protein
Membrane:	Amersham Hybond-P
Blocking:	Amersham ECL Prime blocking agent (2% in PBS-T)
Primary Ab:	Rabbit anti-TAB 1 diluted 1:3000
Secondary Ab:	HRP conjugated anti-rabbit IgG diluted 1:100 000
Imaging:	ImageQuant LAS 4000 mini
Exposure time:	3 min

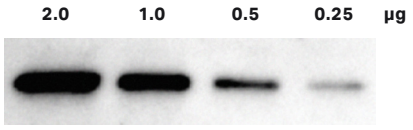

Fig 10. Western blotting detection of endogenous TAB 1 in 293 T cell lysate. The high signal intensity of Amersham ECL Select gives bright bands and high sensitivity.

Amersham ECL Select allows you to detect the bands that make a difference

Amersham ECL Select produces a very high signal intensity, which gives strong and bright protein bands with low background, resulting in high sensitivity. In a side-by-side comparison with other commercially available chemiluminescent reagents, Amersham ECL Select showed excellent performance (Fig 11).

Fig 11. Detection of ERK ½ in HeLa cell lysate. Comparative performance evaluation of Amersham ECL Select, Luminata Forte (Millipore), SuperSignal™ West Femto, and SuperSignal West Dura (Thermo Scientific). All blots were performed in triplicates according to their manufacturer's instructions.

Ordering information

Product	Code no.
Amersham ECL Select Western Blotting Detection Reagent for 1000 cm^2 membrane*	RPN2235

Related products†

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

ImageQuant LAS 4000	28-9558-10
Amersham Hyperfilm Blue, 18 × 24 cm (100 sheets)	28-9888-21
Amersham Hyperfilm ECL, 18 × 24 cm (50 sheets)	28-9068-36

* Includes Solution A (luminol solution, 50 ml) and Solution B (peroxide solution, 50 ml)
† For a more comprehensive list of related products, please refer to www.cytiva.com/ecl

cytiva.com/eclselect

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