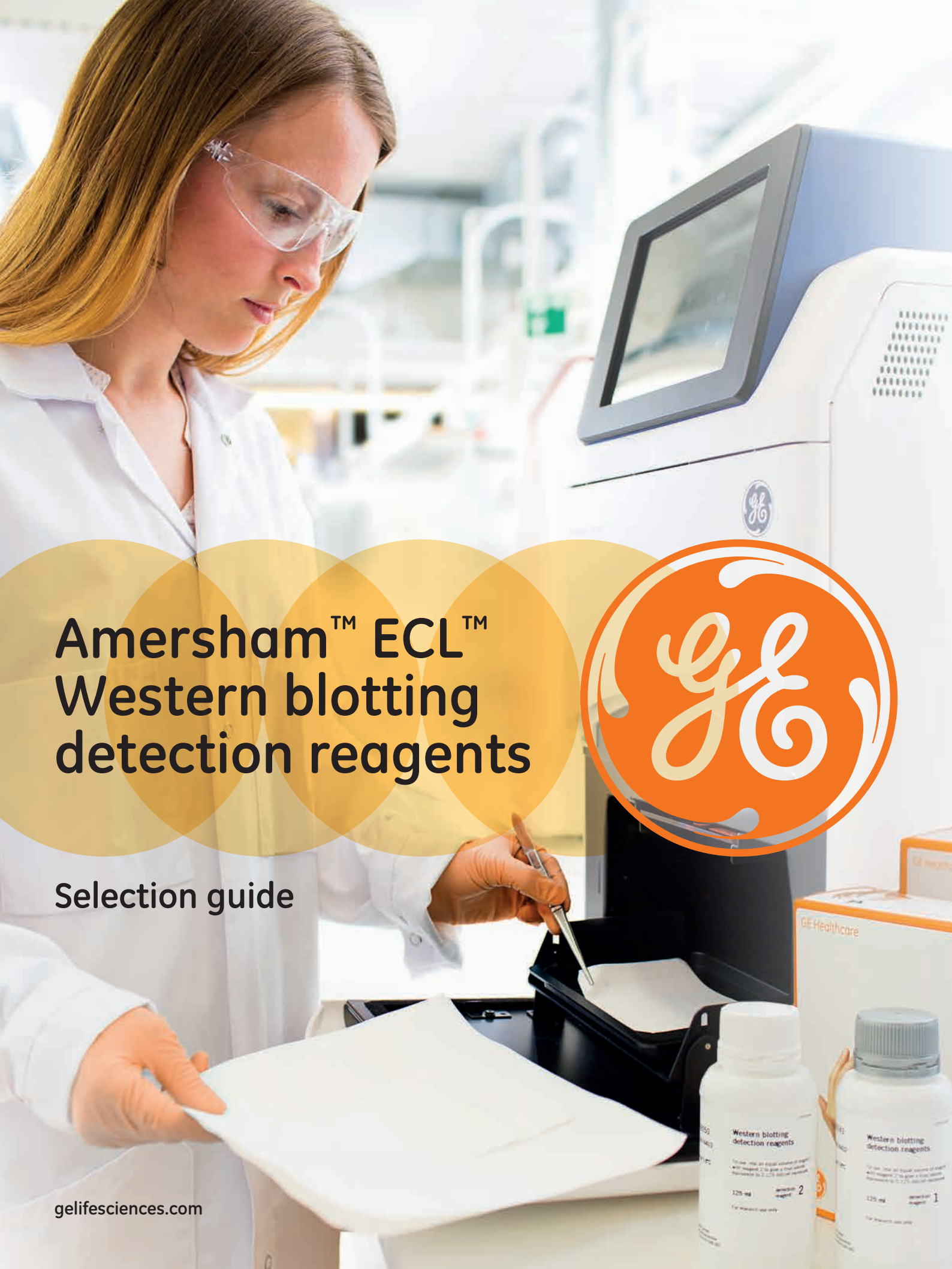


Amersham™ ECL™ Western blotting detection reagents

Selection guide

gelifesciences.com

Choose the ECL Western blotting detection reagent that best suits your needs

Enhanced chemiluminescent (ECL) detection is based on antibodies conjugated to horseradish peroxidase (HRP). HRP catalyzes the oxidation of luminol in the presence of peroxide, generating emission of low intensity light at 428 nm. The signal intensity is dependent of the number of HRP molecules, and accordingly proportional to the amount of antibody bound to the target molecule (Fig 1).

Since the early 1990s, GE Healthcare Life Sciences has continuously developed enhanced chemiluminescence detection systems, which today are among the most widely used detection reagents for Western blotting applications. GE Healthcare offers a variety of chemiluminescence detection reagents, with the best choice depending on the aim of the experiment.

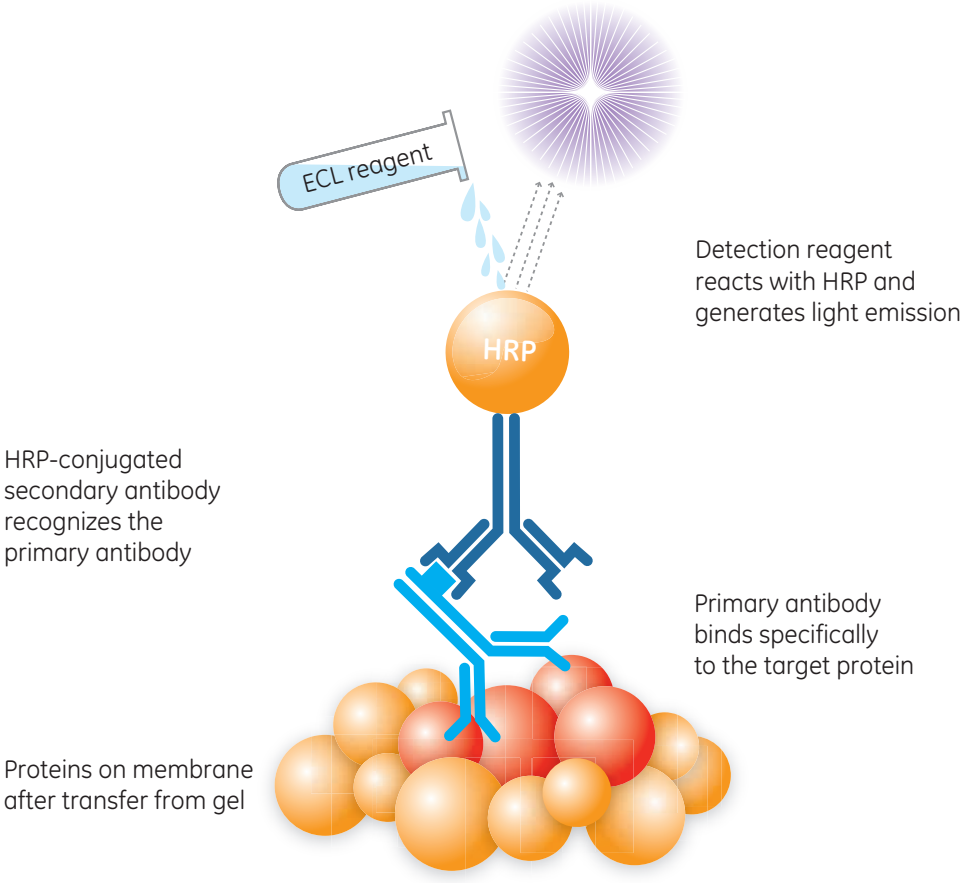
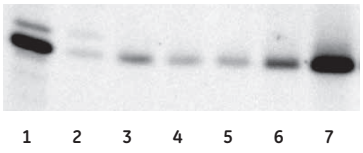


Fig 1. The principle of chemiluminescent Western blotting detection.

Amersham ECL

- One of the first commercially available chemiluminescent detection reagents
- Entry-level chemiluminescent detection reagent
- Reagent of choice for confirmatory Western blotting applications

Western blotting is a valuable tool for verifying identity of recombinantly expressed proteins, as well as for identification of protein fractions to be used for further enrichment of the target molecule (Fig 2).



Lane 1: start material, 3 µl
Lane 2: flow through, 3 µl
Lane 3: wash 1, 3 µl
Lane 4: wash 2, 3 µl
Lane 5: wash 3, 3 µl
Lane 6: eluate 1, 3 µl
Lane 7: eluate 2, 3 µl

Fig 2. Amersham ECL Western blotting detection reagent used for confirmation of histidine-EGFP, recombinantly expressed in Sf21 insect cells and enriched using Mag Sepharose™ magnetic beads.

Amersham ECL Prime

- A highly sensitive chemiluminescent Western blotting detection reagent
- Intense and long lasting signal, enabling large experimental series
- Accurate detection of both high and low abundant proteins on the same blot

Amersham ECL Prime is a highly sensitive detection system characterized by an extremely stable signal emission, allowing for repeated exposures and facilitating processing of several blots in the same experimental run. In addition, the high intensity of the emitted signal makes Amersham ECL Prime suitable for detection of low abundance proteins (Fig 3).

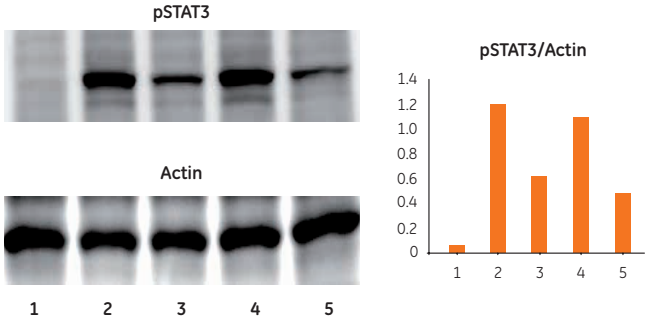


Fig 3. Western blotting detection of Tyr705-phosphorylated STAT3 (pSTAT3) in five different HeLa cell lysates. In each sample, the target protein was quantified relatively to actin levels in the same lysate. The target protein was detected using Amersham ECL Prime.

Amersham ECL Select™

- The most sensitive chemiluminescent Western blotting detection reagent in the Amersham ECL product range
- Exceptional signal intensity, resulting in bright, clear bands even at low protein levels
- Offers high sensitivity even when using highly diluted primary and secondary antibodies

The high signal intensity makes Amersham ECL Select reagent suitable for the most demanding Western blotting applications, including detection of minute protein quantities (Fig 4). The high sensitivity and the broad linear dynamic range of Amersham ECL Select enables qualitative as well as quantitative analyses. Amersham ECL Select reagent generates very intense light emission, allowing for use with highly diluted antibodies in your experiments.

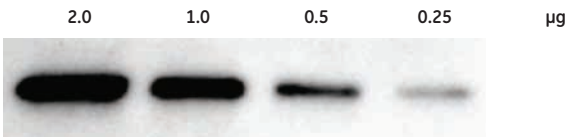
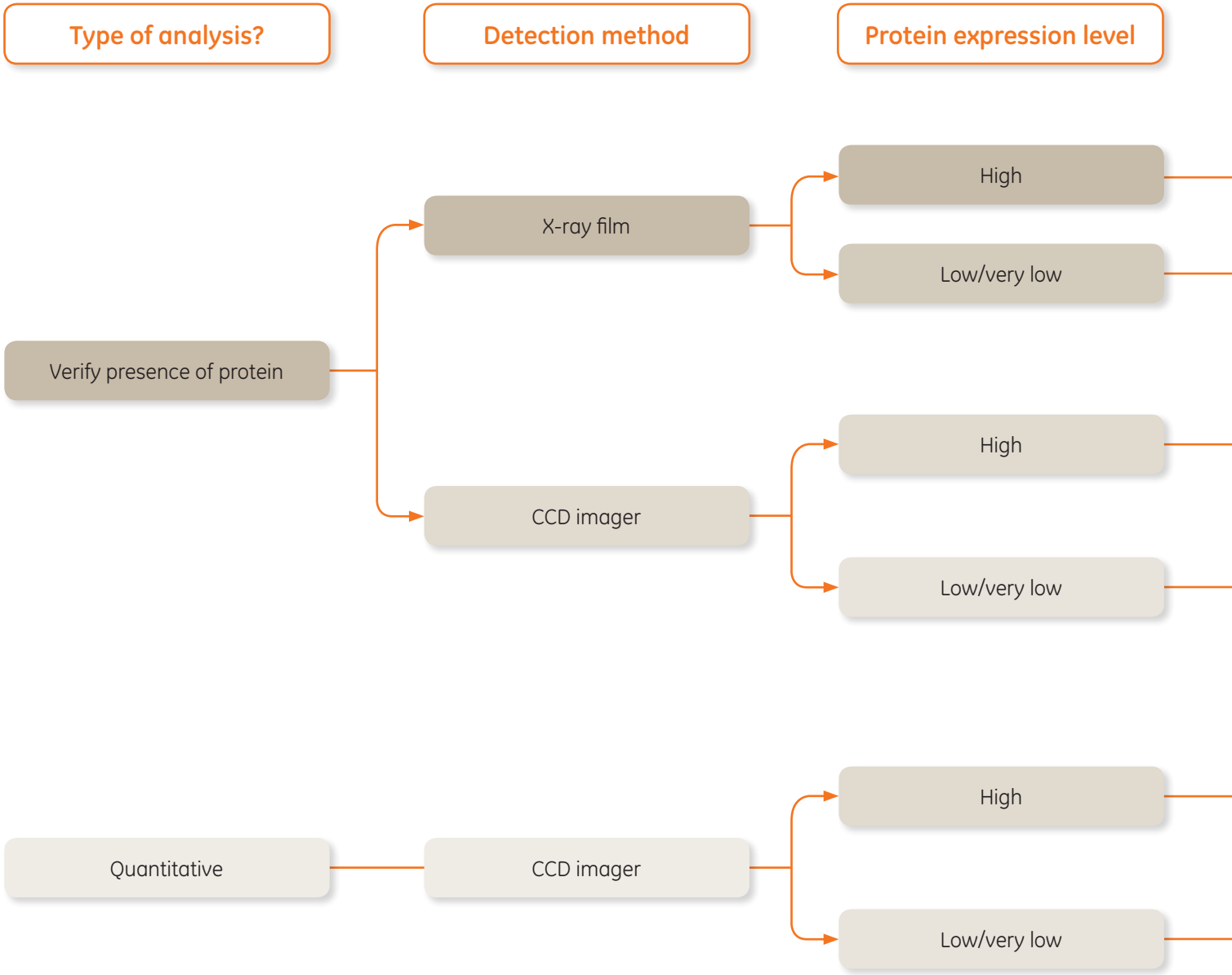


Fig 4. Western blotting detection of endogenous TAB 1 in 293 T cell lysates. The high signal intensity of Amersham ECL Select generates bright bands and offers highly sensitive detection.

Selection guide—Amersham ECL Western blotting detection reagents

Choose a detection reagent depending on the purpose of your experiment



Experimental characteristics	Recommended detection reagent
	Amersham ECL
	Amersham ECL Select
	Amersham ECL
Large experimental setup	Amersham ECL Prime
Scarce antibodies	Amersham ECL Select
	Amersham ECL
Large experimental setup	Amersham ECL Prime
Scarce antibodies	Amersham ECL Select

Benchmarking data

Amersham ECL

Figure 5 shows a side-by-side comparison of Amersham ECL reagent with other chemiluminescence detection reagents in the same sensitivity range.

Samples: Two-fold dilution series of HeLa cell lysate starting at 5 µg
Primary antibody dilution: 1:1000
Secondary antibody dilution: 1:10 000
Detection: Amersham Imager 600, 1 min exposure

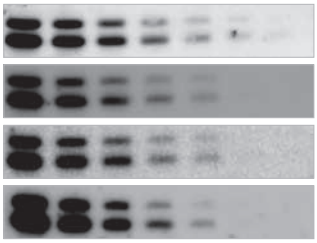


Fig 5. Amersham ECL reagent exhibits similar sensitivity as the competitor reagents in Western blotting detection of ERK 1/2.

Amersham ECL Prime

Figure 6 shows a side-by-side comparison of Amersham ECL Prime with other high-sensitive chemiluminescence detection reagents.

Samples: Two-fold dilution series of HeLa cell lysate starting at 10 µg
Primary antibody dilution: 1:5000
Secondary antibody dilution: 1:30 000
Detection: Amersham Imager 600, 75 s exposure

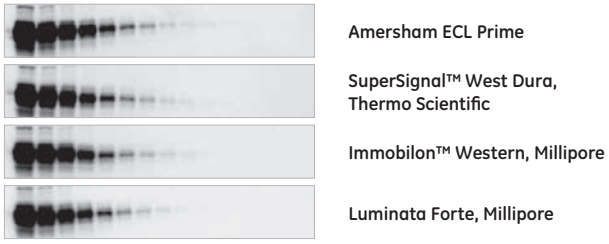


Fig 6. Amersham ECL Prime exhibits similar sensitivity as the competitor reagents in Western blotting detection of ERK 1/2. The Millipore reagents were slightly less sensitivity and also reached saturation at the highest concentrations (10 to 5 µg), decreasing the dynamic range of these reagents.

Figure 7 shows Amersham ECL Prime in comparison with Clarity™ Western ECL Substrate from Bio-Rad.

Samples: Two-fold dilution series of HeLa cell lysate starting at 2.5 µg
Primary antibody dilution: 1:3000
Secondary antibody dilution: 1:30 000
Detection: Amersham Imager 600, 3 min exposure

Fig 7. Amersham ECL Prime exhibits higher sensitivity and signal intensity compared with Clarity Western ECL Substrate in Western blotting detection of ERK 1/2.

Amersham ECL Select

Figure 8 shows a side-by-side comparison of Amersham ECL Select with other high-sensitive chemiluminescence detection reagents.

Samples: Two-fold dilution series of HeLa cell lysate starting at 2.5 µg
Primary antibody dilution: 1:10 000
Secondary antibody dilution: 1:100 000
Detection: Amersham Imager 600, 3 min exposure

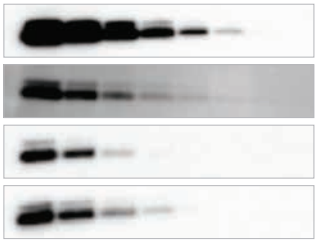


Fig 8. Amersham ECL Select shows brighter bands, lower background, and higher limit of detection compared with competitor reagents in Western blotting detection of ERK 1/2.

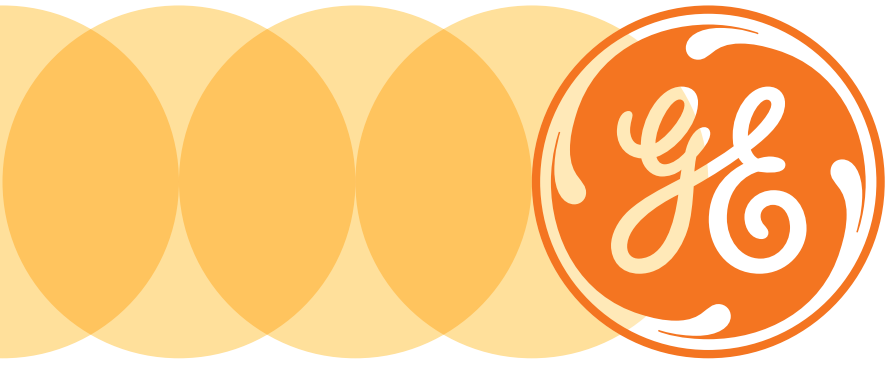
Ordering information

Product	Quantity	Code number
Western blotting detection reagents		
Amersham ECL Western Blotting Detection Reagents	For 1000 cm^2 membrane	RPN2109
Amersham ECL Western Blotting Detection Reagents	For 2000 cm^2 membrane	RPN2209
Amersham ECL Western Blotting Detection Reagents	For 4000 cm^2 membrane	RPN2106
Amersham ECL Western Blotting Detection Reagents	For 6000 cm^2 membrane	RPN2134
Amersham ECL Prime Western Blotting Detection Reagents	For 1000 cm^2 membrane	RPN2232
Amersham ECL Prime Western Blotting Detection Reagents	For 3000 cm^2 membrane	RPN2236
Amersham ECL Select Western Blotting Detection Reagents	For 1000 cm^2 membrane	RPN2235
Amersham ECL Western Blotting Reagent Pack (Includes detection reagent, secondary antibodies, and blocking agent)	For at least 10 miniblots	RPN2124

Western blotting membran blocking reagent	Quantity	Code number
Amersham ECL Prime Blocking Reagent	For at least 20 miniblots	RPN418
Amersham ECL Blocking Agent	For at least 20 miniblots	RPN2125

HRP conjugated secondary antibodies	Quantity	Code number
Amersham ECL HRP Anti-Mouse Secondary antibody	100 µl	NA931-100UL
Amersham ECL HRP Anti-Mouse Secondary antibody	1 ml	NA931-1ML
Amersham ECL HRP Anti-Rabbit Secondary antibody	100 µl	NA934-100UL
Amersham ECL HRP Anti-Rabbit Secondary antibody	1 ml	NA934-1ML
Amersham ECL HRP Anti-Rat Secondary antibody	1 ml	NA935

The products presented in this brochure are available from GE Life Sciences distributors www.gelifesciences.com/distributors



www.gelifesciences.com/amershamwb

GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
29-1034-92 AA 03/2014