Read this First
Important information!
Amersham™ ECL™ Prime

Code: RPN2232

Warning
For research use only.
Not recommended or intended for diagnosis of disease in humans or animals.
Do not use internally or externally in humans or animals.

Note

These are additional notes and are intended to be used in conjunction with the main product instruction booklet for Amersham ECL Prime.

For handling, storage, expiry and safety warnings and precautions, please refer to the main product literature.

Amersham ECL Prime

Amersham ECL Prime is a highly sensitive enhanced chemiluminescence reagent for detection of horseradish peroxidase (HRP) conjugates on Western blots. The Amersham ECL Prime reagent is developed to provide an extremely high signal with improved duration by the addition of a specific catalyst for an increased light output. The enhanced oxidation of luminol is a multi-step reaction regulated by the HRP amount. To obtain optimal sensitivity and signal duration when using Amersham ECL Prime it is important to use higher dilutions of the antibodies compared to what typically is recommended.

Amersham ECL Prime requires less sample and less primary and secondary antibodies than other reagents such as Amersham ECL and our former Amersham ECL Plus.

Note the following recommendations when changing from Amersham ECL Plus or similar reagents.

Primary antibody
Increase dilution of primary antibody 2 to 4 times.

Recommended dilution range is **1:3000–1:7000.**

Secondary antibody
Increase dilution of secondary antibody 2 to 4 times.

Recommended dilution range is **1:30 000–1:70 000**.

Dare to dilute!
It is critical to use higher antibody dilutions.

Washing
Wash membrane 6 times in at least 50 ml washing buffer for 5 min each.

Legal

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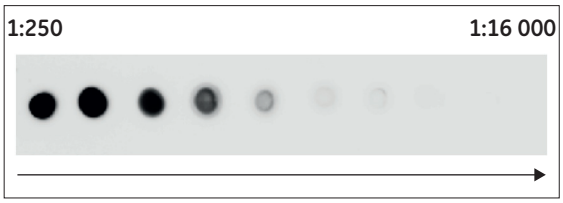
Troubleshooting

Problem	Possible cause	Action
Weak or no signal	Too much HRP in the reaction causes too fast and too strong of a reaction, resulting in substrate depletion and a quickly fading, or non-captured signal.	Reduce HRP in the reaction by decreasing sample amount and/or by diluting antibodies further.
	Non-detectable or too low amount of target protein.	Increase sample amount.
	Weak or nonfunctional reagent.	Test reagent functionality with the blue light test or a dot blot test. **Blue light test** - In a dark room, add 2 µl of the HRP conjugate to 1 ml of detection reagent working solution (solution A and solution B in 1:1 ratio) in a small test tube. You should see a blue light flash. **Dot blot** - Dilute the HRP conjugate in a 2-fold dilution series starting at a dilution of 1:250 (make 6 to 10 dilutions). Apply 1 µl of each dilution in a row on a nitrocellulose membrane. Let dry for 10 min. Add 1 to 3 ml of detection reagent working solution (solution A and solution B in 1:1 ratio) and capture the signal using X-ray film or CCD camera. A functional reagent should give detectable spots (Fig 1).  **Fig 1.** Illustration of a dot blot membrane. Membrane with a 2-fold dilution series of HRP conjugate (HRP-conjugated anti-rabbit IgG NA934, GE Healthcare) starting at a dilution of 1:250. Amersham ECL Prime was used as detection reagent and signal capture was performed using ImageQuant™ LAS 4000.
High or speckled background	Too much protein or antibody.	Dilute antibodies further, especially the secondary antibody.
	Insufficient washing.	Add additional washing steps and use large volumes of washing buffer.
	Non-dissolved blocking agent or aggregates in antibody solutions.	Dissolve blocking agent completely. Use fresh antibody solution and filter through a 0.2 µm filter if necessary.
Fading signal	Too much HRP causes an imbalance in the chemiluminescence reaction, which results in too fast and too strong of a reaction that causes substrate depletion resulting in a fading signal.	Reduce HRP in the reaction by decreasing sample amount and/or by diluting antibodies further (Fig 1).
White “ghost” band	Substrate depletion caused by too much HRP in the reaction.	Reduce HRP in the reaction by strongly decreasing the sample amount and by diluting antibodies further.
Brownish band on the membrane (“burned” membrane)	Extremely strong chemiluminescence reaction caused by an extremely large amount of HRP.	Reduce HRP substantially in the reaction by strongly decreasing sample amount and by increasing antibody dilutions.

imagination at work