Amersham™ ECL™ Prime

Instructions for Use

Introduction

Product code

RPN2232

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and not to be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

The information provided in this document is intended to be used in conjunction with *Amersham™ ECL™ Prime Western Blotting Detection Reagent Product booklet 28982942*.

For handling, storage, expiry, and safety warnings and precautions, refer to *Amersham ECL Prime Western Blotting Detection Reagent Product booklet 28982942*.

Amersham ECL Prime

Amersham ECL Prime is a high sensitive enhanced chemiluminescence reagent for detection of horseradish peroxidase (HRP) conjugates on Western blots. Amersham ECL Prime is developed to provide a high signal with improved duration, by the addition of a specific catalyst that increases with 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.

See the table below for recommended antibody dilution range.

Antibody	Recommended dilution range
Primary antibody	1:3000 to 1:7000
Secondary antibody	1:30 000 to 1:70 000

Washing

After incubation with the antibody, wash the membrane six times in > 50 mL washing buffer for five minutes each time.

Note: *For a detailed overview of the protocol, refer to the protocol chapter of Amersham ECL Prime Western Blotting Detection Reagent Product booklet 28982942.*

Instructions

Follow the steps below for preparation and use of the reagent.

Detection

Step	Action
1	Prepare a working solution of detection reagent by mixing detection solutions A (luminol) and B (peroxide) in a ratio of 1:1. The final volume of detection reagent required is 0.1 mL/cm² membrane. ***Note:*** *If the working solution of detection reagent is not to be used immediately, protect it from exposure to light.*
2	Drain the excess washing buffer from the washed membrane and place it with the protein side up in a suitable container or on a sheet of plastic wrap or other suitable clean surface.
3	Add the prepared working solution detection reagent onto the membrane and make sure it completely covers the membrane.
4	Incubate for 5 minutes at room temperature.
5	Drain off excess detection reagent by holding the membrane edge gently against a tissue.

Image analysis

Refer to the product booklet for instructions on image analysis.

Troubleshooting

Problem	Possible cause	Action
Weak or no signal	Too much HRP in the reaction causes too fast and strong reaction, resulting in substrate depletion and a quickly fading, or non-captured signal.	Reduce HRP in the reaction by decreasing amount of sample 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 a Blue light test or a Dot blot test. For details see sections *Blue light test and Dot blot test in sections below.*

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Problem	Possible cause	Action
High or speckled background	Too much protein or antibody.	Dilute antibodies further, especially the secondary antibody.
	Insufficient washing.	Add additional washing steps and use larger volumes of washing buffer.
	Non-dissolved blocking agent or aggregates in antibody solutions.	Dissolve the 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 a too fast and strong reaction that causes substrate depletion, resulting in a fading signal.	Reduce HRP in the reaction by decreasing the amount of sample and/or by diluting antibodies further.
White "ghost" band	Substrate depletion caused by too much HRP in the reaction.	Reduce HRP in the reaction by strongly decreasing the amount of sample 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 the amount of sample and by increasing antibody dilutions.

Blue light test

In a dark room, add 2 µL of the HRP conjugate to 1 mL of detection reagent working solution in a small test tube.

If the detection reagent is functional, a blue light flash is observed.

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Dot blot test

See the steps below to perform a dot blot test.

Step	Action
1	Dilute the HRP conjugate in a twofold dilution series starting at a dilution of 1:250. Make 6 to 10 dilutions.
2	Apply 1 µL of each dilution in a row on a nitrocellulose membrane and let it dry for 10 minutes.
3	Add 1 to 3 mL of detection reagent working solution and capture the signal using Xray film or a CCD camera. *Result:* A functional reagent must give detectable spots.

The image below shows a dot blot membrane with a twofold dilution series of HRP conjugate (HRP-conjugated anti-rabbit IgG NA934, Cytiva), starting at a dilution of 1:250. Amersham ECL Prime was used as detection reagent and signal capture was performed using ImageQuant™ LAS 4000.



Related products

For more information on products, pack size, and information on additional products, visit *cytiva.com/westernblotting*.

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29032061 AE V:9 12/2022