Easy tab ECL prime tablets

Instructions for Use

Introduction

Product code

RPN2237

Important

Read these instructions carefully before using the product.

Intended use

The product is intended for research use only, and shall not 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 respective *Safety Data Sheets*.

Components

The product is comprised of the following components:

Name	Description	Quantity
Tablet A	Luminol derivative/ enhancer tablet	20 tablets
Tablet B	Peroxide tablet	20 tablets

Storage and expiry

Refer to the label on the product packaging for storage conditions and expiry date.

Background

Product overview

Easy tab ECL prime tablets are tablets used to produce a high sensitive enhanced chemiluminescence reagent for detection of horseradish peroxidase (HRP) conjugates on Western blots. The reagent working solution provides a high signal with a long 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.

Dilution ranges

To obtain optimal sensitivity and signal duration when using Easy tab ECL prime tablets, it is important to optimize the dilutions of the antibodies.

See the table below for recommended antibody dilution ranges.

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

Note: *Dilution ratios must be optimized for the specific application. For additional recommendations, refer to documentation from the antibody manufacturer.*

Washing

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

Protocol

Required materials

The following materials must be supplied by the user to perform the detection with Easy tab ECL prime tablets.

- Container for the working solution, such as a 15 mL centrifuge tube
- Deionized water
- Washing buffer
- Membrane with protein transferred and antibody incubation and washing performed.
- Plastic wrap (optional)
- Reaction folder (optional)
- CCD imaging system

Detection

Step	Action
1	Prepare a working solution of detection reagent by adding tablet A (luminol) and tablet B (peroxide) to 5 mL of deionized water in a suitable container. ***Note:*** *5 mL is a sufficient volume for two 8×9 cm membranes.*
2	Wait for the tablets to be fully dissolved, which takes less than ten minutes when placing the container in an end-over-end mixer or on a tilting or rotating table. ***Note:*** *If the working solution of detection reagent is not to be used immediately, protect the solution from exposure to light.*
3	Drain the excess washing buffer from the washed membrane and place it with the protein-side up in a suitable container, on a sheet of plastic wrap, or on another suitable clean surface.
4	Pipette the working solution over the entire membrane surface.

Step	Action
5	Incubate for 5 minutes at room temperature.
6	Drain off excess detection reagent by holding the membrane edge gently against a tissue.
7	*Optional:* Cover the membrane with a piece of plastic cut to size, such as a section of a reaction folder. This prevents the membrane from drying during imaging.
8	Use a CCD imaging system to analyze the membrane.

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*.
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.

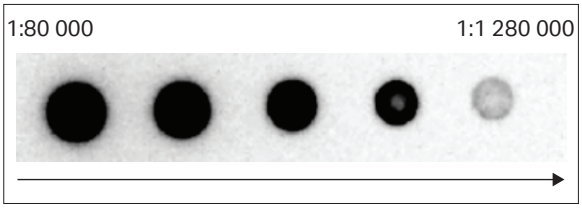
If the detection reagent is functional, a blue light flash is observed.

Dot blot test

Follow 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 1:40 000. Make 6 to 10 dilutions.
2	Apply 2 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 X-ray 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 1:80 000 and 2 µL added to a dry NC membrane. The Easy tab ECL prime tablets were used as the detection reagent, and the signal capture was performed using ImageQuant™ 800.



Related products

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