Amersham **ECL** detection reagents

Product Specification Sheet

Introduction

Product codes

RPN2105

RPN3004

About

For use with ECL™ nucleic acid labelling and detection systems.

Important

Read these instructions carefully before using the products.

Intended use

The products are 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 Safety Data Sheets.

This product contains one or more chemical substances supplied in small quantities. In the form supplied, these substances are not classified as dangerous within the meaning of the definitions of the Council of European Communities Directive 67/548/EEC and subsequent amendments.

You are reminded that certain components in the solutions may cause bleaching on contact with skin.

Storage

Store refrigerated at 2°C to 8°C. Under these conditions the product is stable for at least 3 months.

Expiry

See outer packaging.

Components

RPN2105:

Sufficient for 4000 cm^2 membrane:

250 mL detection reagent 1

250 mL detection reagent 2

RPN3004:

Sufficient for 2000 cm^2 membrane:

125 mL detection reagent 1

125 mL detection reagent 2

Background

ECL detection reagents may be used for enhanced chemiluminescent, non-radioactive detection of nucleic acid blots which have been labelled with Horseradish Peroxidase. ECL detection has been successfully used in Southern, Northern, colony/plaque and dot blotting applications (3–8). ECL detection is particularly useful for the analysis of PCR[1] amplified material. There are two detection reagents that should be mixed immediately before use. Detection reagent 1 decays to Hydrogen Peroxide, the substrate for peroxidase. Reduction of Hydrogen Peroxide by the enzyme is coupled to the light producing reaction by detection reagent 2. This contains luminol, which on oxidation produces blue light. The light output is increased and prolonged by the presence of an enhancer so that it can be detected on a blue-light sensitive film (See Figure 1 below).

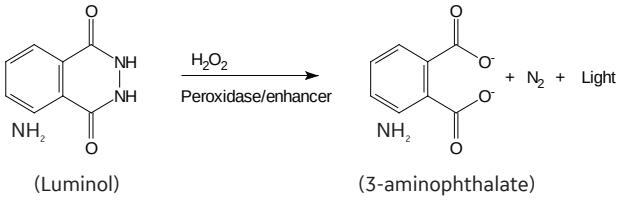

Fig 1. The ECL reaction

Specification

Lambda *Hind III* Southern blots on Hybond™ -N+ membrane, are hybridized overnight at 42°C with lambda *Hind III* DNA probe at 10 ng/mL which has been labelled using the ECL direct labelling system. After the washes, the detection reagents (RPN3004, RPN2105) are used in the detection step.

To pass the QC specification the 4.36 kb band on the 10 pg track of lambda *Hind III* must be visualized on a 30 minute exposure to Hyperfilm™-ECL or Hyperfilm-MP.

Protocol for ECL detection on membranes

Note: *Read through this whole section before proceeding. It is necessary to work quickly once the blots have been exposed to the detection solutions. All steps can be carried out in a dark room; it is only necessary to switch off the light after step 5.*

[1] PCR, the polymerase chain reaction, is a patented process of Hoffman-la Roche, US patent 4683202.

Equipment that is needed includes an X-ray film cassette, a roll of SaranWrap (other 'cling-films' may be suitable), a timer and autoradiography film, for example Hyperfilm-ECL (RPN2103K).

Step	Action
1	Take the detection reagents that are supplied and mix an equal volume of detection solution 1 with detection solution 2 to give sufficient to cover the blot. ***Note:*** *The final volume required is 0.125 mL/cm^2 membrane.*
2	Remove excess wash buffer by placing blots on paper towels or 3 MM paper for 1 minute. Transfer blots to fresh containers. Add the mixed detection reagents directly to the blots on the face carrying the DNA. ***Note:*** *Care should be taken to use a fresh pipette tip for every measurement and not to confuse the tops of any reagent containers, as cross-contamination may give spurious results and will significantly reduce the working life of the reagents.*
3	Incubate for precisely 1 minute at room temperature. ***Note:*** *An alternative method is to equilibrate the drained blots in a half quantity of solution 2 (0.0625 mL/cm^2), add to this a similar quantity of solution 1, and mix by gentle shaking.*
4	Drain off excess detection buffer and wrap blots in SaranWrap. Gently smooth out air pockets.
5	Place the blots, DNA side up, in the film cassette. Work as quickly as possible; minimize the delay between incubating the blots in substrate and exposing them to the film (next step). ***Note:*** *Ensure that there is no free detection reagent in the film cassette; the film must not get wet.*
6	Switch off the lights and place a sheet of autoradiography film (for example Hyperfilm-ECL) on top of the blots, close the cassette and expose for 1 minute. ***Note:*** *Do this in a dark-room, using red safelights. Do not move the film while it is being exposed. Hyperfilm-ECL can be pre-flashed if required.*
7	Remove film, immediately replace with a fresh piece of unexposed film, and reclose film cassette. Start timer. ***Note:*** *Develop first piece immediately, and on the basis of its appearance estimate how long to continue the exposure of the second piece of film. Second exposures can vary from 10 minutes to several hours, this will depend to some extent on the amount of target nucleic acid on the blot.*
8	Develop second piece of film after suitable exposure time.
9	Interpret results.

Related products

Hyperfilm-ECL	
Pack of 25 sheets, 18 x 24 cm	RPN2103K
Pack of 25 sheets, 30 x 40 cm	RPN2104K
Pack of 25 sheets, 5 x 7 inches	RPN1674K
Pack of 25 sheets, 10 x 12 inches	RPN1681K

Hybond-N+ and Hybond-ECL
Part of an extensive range of membranes available from Cytiva.

ECL random prime labelling and detection systems	
Based on the incorporation of a hapten (Fluorescein) with detection by ECL	
30 labelling reactions;	
ECL detection reagents for 2000 cm^2 membrane	RPN3030
60 labelling reactions;	
ECL detection reagents for 4000 cm^2 membrane	RPN3031

ECL direct nucleic acid labelling and detection system	
Labelling reagents for 5 µg DNA	
Hybridization buffer and detection reagents or 2000 cm^2 membrane	RPN3000
Labelling reagents for 10 µg DNA	
Hybridization buffer and detection reagents for 4000 cm^2 membrane	RPN3001

ECL 3'-oligolabelling and detection system	
For tailing oligos with Fluorescein-dUTP	
Sufficient for 500 pmols oligonucleotide	
ECL detection reagents for 4000 cm^2 membrane	RPN2131

ECL 5'-thiol oligolabelling system	
For the direct labelling of thiol modified oligonucleotides with Horseradish Peroxidase with detection by ECL	
Sufficient for 5 x 5 µg oligonucleotide	
ECL detection reagents for 4000 cm2 membrane	RPN2111

ECL probe-amp reagents	
6 reactions	RPN3020
12 reactions	RPN3021

References

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29460174 AA V:3 01/2021

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