

Amersham ECF substrate for Western blotting

Product Specification Sheet

Introduction

Product code

RPN5785

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.

Storage and stability

Upon receipt the reagents should be stored at 2–8°C. In unreconstituted form, the reagents are stable for at least 3 months. Once reconstituted, the ECF substrate can be stored at 2–8°C for 2–4 weeks. For longer storage, we recommend that it is divided into aliquots and frozen. Repeat freezing and thawing of any aliquot should be avoided.

Components

ECF substrate	36 mg
ECF dilution buffer	60 mL

Description

ECF™ substrate contains substrate and dilution buffer. In the presence of alkaline phosphatase a phosphate group is cleaved from the substrate forming a highly fluorescent product which fluoresces strongly at 540–560 nm. This reagent can be used with the ECF range of products applicable for use with fluorescence scanning instruments, for example Molecular Dynamics FluorImager and Storm.

Detection with ECF substrate

Note: Read through the whole section before proceeding.

Step	Action
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1	Prepare the working stock of ECF substrate by adding the 60 ml of ECF dilution buffer, supplied, to the 36 mg of ECF substrate. Mix the bottle on a roller mixer until completely dissolved.
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Note:

Avoid the use of stir bars or any other mechanical devices.

Step	Action
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2	Calculate the volume of ECF substrate required to cover the membrane using 24 µL ECF substrate/cm ² membrane. Place a piece of SaranWrap on the bench and smooth out, ensuring no air bubbles or creases exist. Pipette the required volume of ECF substrate on to the SaranWrap, drain the blot of wash buffer and then lay the blot, protein side down, on to the solution. Leave to incubate for 5 minutes at room temperature.
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Note:

The ECF substrate must be pipetted on to a flat surface to ensure it spreads evenly across the membrane. Wearing gloves handle the membranes carefully by the edges with blunt non-serrated forceps. ECF substrate incubation can be extended for up to 20 minutes to increase the signal obtained. The blot should not be moved in this period. Ensure no air bubbles are trapped beneath the membrane during the incubation step.

3	Place the membrane directly on to the sample holder of the fluorescence scanning instrument and place a clean glass plate on top of it to keep it in place. Scan using a 570 nm filter.
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Note:

Refer to the scanner operating manual for full instructions. From the initial scan the PMT voltage can be varied to give the best image.

Related products

ECF Western blotting reagent pack (mouse antibodies)	
Sufficient for 2500 cm ² of membrane	RPN5781
Sufficient for 5000 cm ² of membrane	RPN5782
ECF Western blotting reagent pack (rabbit antibodies)	
Sufficient for 2500 cm ² of membrane	RPN5783
Sufficient for 5000 cm ² of membrane	RPN5784
ECF Western blotting kit	
Sufficient for 2500 cm ² of membrane	RPN5780
Range of fluorescein, PbxI and CyDye™ conjugated secondary antibodies for direct detection	See catalogue for details
Full Range Rainbow™ protein molecular weight markers	RPN800
Hybond™ P (PVDF)	
Pack of 10 sheets, 20 x 20 cm	RPN2020F
Roll of membrane, 20 cm x 3 m	RPN303F
Hybond ECL™ (nitrocellulose)	
Pack of 10 sheets, 20 x 20 cm	RPN2020D
50 discs	RPN82D

For further details on these products and for information on products for ECL and ECL Plus detection for Western blotting, see the current Cytiva catalogue or contact your local Cytiva office.

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