

Amersham ECF Substrate

Product Specification Sheet

Introduction

Product code

RPN3685

About

For the chemifluorescent detection of alkaline phosphatase.

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

Store at 2°C to 8°C. Stable for at least 3 months when stored under the recommended conditions.

Component

- 60 mL, ECF detection buffer, contains Diethanolamine
- 36 mg, ECF substrate

Quality control

ECF substrate and ECF detection buffer are tested by Cytiva quality control group in DNA hybridizations using appropriate products from the Gene Images labelling and detection range.

Usage in non-radioactive detection

This product is compatible with all ECF substrate related products within the Gene Images range. Full labelling, hybridization and detection protocols are provided with the relevant products.

Note: Please read through this whole section before proceeding.

Note: Wear powder-free gloves or rinse gloved hands with water before use to remove powder.

Protocol

Step	Action
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| 1 | Pour the entire contents of the bottle containing the detection buffer into the bottle which contains the ECF detection reagent. Screw the top on firmly and shake the bottle gently (for example, on a roller-mixer) for about 10 minutes to fully dissolve the ECF detection reagent |
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Step	Action
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| | <p>Note:
Store the dissolved ECF substrate, in aliquots, at -15°C to -30°C.</p> |
| 2 | <p>Drain off any excess wash buffer from the blots (by touching the corner of the blot against the box used for washing the blots or other convenient clean surface) and place them (sample side up) on a clean, non-absorbent, flat surface.</p> <p>Note:
SaranWrap or a section cut from a Gene Image detection bag (RPN3609) can be used to place the blot upon. Proceed directly to step 3 so that the blots are not allowed to dry out.</p> |
| 3 | <p>Pipette ECF substrate on to the blots (~25 µL/cm²) and incubate for 1 minute. Transfer the blots directly to a fresh detection bag. Fold the plastic over the top of the blots and immediately spread the reagent evenly over the blots.</p> <p>Note:
This can be done either by rolling a 5 mL pipette over the surface or wiping the surface with a gloved hand.</p> |
| 4 | <p>Seal the bag to stop the sample drying out and incubate at room temperature in the dark, for example in a drawer or a film cassette, for the required length of time. The optimal time for your particular system can be found by rescanning at various times.</p> <p>Note:
For high target levels an acceptable scan image may be obtained after 1 hour. Scanning up to 24 hours after addition of substrate will provide a much stronger signal suited to low target applications.</p> |
| 5 | <p>Place the bag containing the blot(s) on to the flat bed, fluorescent scanning instrument.</p> <p>Note:
Ethanol or water placed between the lower surface of the bag and the glass will greatly improve the image obtained.</p> |
| 6 | <p>Scan the blot using an appropriate emission filter as available and according to the guidelines for use of the scanning instrument.</p> <p>Note:
ECF has a broad excitation spectrum with a maximum at 430 nm and an emission maximum at 560 nm.</p> |

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