



ISH Detection Kit

Cat. No. **VP-Y180**

For *in situ* Hybridization to detect fluorescein-conjugated probes

Product Specifications

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1 Introduction

The ISH Detection Kit is intended for the detection of fluorescein-conjugated nucleic acid probes hybridized to their targets in cell or tissue preparations.

The kit can be used in conjunction with a range of fluorescein-conjugated probes. Sufficient reagents for the *in situ* hybridization staining of 100 preparations are provided.

The hybridization is followed by a wash with Tris-buffered saline. Alkaline phosphatase-conjugated Rabbit F(ab') Anti-FITC is then added, and the specimen is incubated at room temperature for 30 minutes followed by a wash. Non-specific binding to the specimen is kept at a minimum due to the use of an antibody F(ab') fragment.

Enzyme substrate (BCIP/NBT) is added and the specimen incubated at room temperature in the dark overnight.

Finally, the specimen is mounted. Positive staining is recognized under the microscope as a dark blue/black color at the site of hybridization.

2 Kit Contents

Vial A Rabbit F(ab') Anti-FITC/AP

Alkaline phosphatase-conjugated antibody to fluorescein isothiocyanate (affinity-isolated rabbit F(ab')). In 100mM Tris buffer, 50mM MgCl₂, 0.1mM ZnCl₂, 4% w/v BSA, stabilizer, preservative, pH 7.5 (0.25ml).

Vial B Enzyme Substrate

(50 x concentrated) 5-bromo-4-chloro-3-indolylphosphate (BCIP) and Nitroblue tetrazolium (NBT). In dimethylformamide solution (0.4ml).

Vial C Inhibitor (Levamisole)

(1000 x concentrated) 1M Levamisole hydrochloride (0.15ml).

3 Materials Required but not Provided

1. General laboratory equipment for histological procedures.
2. Equipment and reagents needed for the hybridization step.
3. VECTABOND™ (Cat. No. SP-1800) treated slides.
4. Tris-buffered saline (TBS): 50mM Tris/HCl, 150mM NaCl, pH 7.6.
5. TBS, 0.1% v/v Triton X-100.
6. TBS, 3% w/v BSA, 0.1% v/v Triton X-100, (conjugate diluent).
7. TBS, 3% w/v BSA, 0.1% v/v Triton X-100, 20% v/v normal rabbit serum.
8. Normal rabbit serum (S-5000).
9. 100mM Tris/HCl, 50mM MgCl₂, 100mM NaCl pH 9.0 (alkaline phosphatase substrate buffer).
10. Aqueous mountant.

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4 Preparation of Reagents

Anti-FITC/AP Working Solution

Dilute Rabbit F(ab') Anti-FITC/AP (Vial A) 1:100 to 1:200 in TBS, 0.1% v/v Triton X-100, 3% w/v BSA.

Enzyme Substrate with Inhibitor

Dilute Enzyme Substrate (Vial B) 1:50 in 100mM Tris/HCl, 50mM MgCl₂, 100mM NaCl pH 9.0. Add 1 µl of Inhibitor (Levamisole) (Vial C) to each ml of diluted Enzyme Substrate.

5 Precautions and Safety

Due to the small volumes in the stock reagent Sarstedt vials, please ensure that each reagent is in the vial and not the vial cap before opening. This can be achieved simply, by either centrifugation or gently tapping the side of the vial.

The Enzyme Substrate contains BCIP, NBT and dimethylformamide. The undiluted reagent is harmful by inhalation or in contact with skin and may be a possible carcinogen. The Inhibitor contains levamisole hydrochloride. The undiluted reagent is harmful by inhalation, in contact with skin or if swallowed.

6 Storage

The reagents of the kit should be stored in the dark at 2-8 °C.

7 Procedure

To ensure that sections adhere to the slides throughout the procedure, it is recommended to use VECTABOND™ treated slides.

Detection

1. Place slides in an incubation tray and cover sections with 100µl of TBS, containing 3% w/v BSA, 0.1% w/v Triton-X-100 and 20% w/v normal rabbit serum. Incubate for 10 minutes.
2. Tip off the blocking solution and add Rabbit F(ab') Anti FITC/AP diluted 1:100 to 1:200 in TBS, 3% w/v BSA, 0.1% w/v Triton X-100. Incubate for 30 minutes.
3. Wash slides in TBS for 2 x 3 minutes.
4. Wash slides in alkaline phosphatase substrate buffer pH 9.0 for 5 minutes.
5. Place slides in incubation tray and demonstrate alkaline phosphatase activity by covering sections with the following solution: Dilute Enzyme Substrate 1:50 in 100mM Tris/HCl, 50mM MgCl₂, 100mM NaCl, pH 9.0. Add 1µl of Inhibitor (Levamisole) to each ml of diluted Enzyme Substrate. Incubate at room temperature in the dark overnight.
6. Wash in running water for 5 minutes.
7. Counterstain in Vector® Hematoxylin QS (H-3404) for 10 seconds maximum (optional step).
8. Mount in aqueous mountant.