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# Product Information

**Anti-FITC-Alkaline Phosphatase**
produced in rabbit, IgG fraction of antiserum

Catalog Number **A5719**
Store at 2-8 °C

## Product Description

Anti-FITC-Alkaline Phosphatase has been developed for the detection of FITC-labeled nucleic acid probes in nylon membrane-based blotting applications. The conjugate is stored in Sigma's Alkaline Phosphatase Storage Buffer to guarantee stability. The conjugate should be diluted 1:100 to 1:200 for colorimetric applications and 1:1000 to 1:2000 for chemiluminescent applications. The use of this conjugate has been optimized in conjunction with Sigma's 10X Blocking Buffer, Catalog Number B6429. Both reagents are components of the CDP-Star Universal Alkaline Phosphatase Detection Kit, Catalog Number UALK. Anti-FITC is developed in rabbit using FITC-BSA as immunogen. Anti-FITC antibody is an IgG fraction of pooled antiserum. The BSA specific antibodies are removed by adsorption on immobilized BSA. The product is prepared from a solution of rabbit anti-FITC conjugated to alkaline phosphatase (calf intestine).

## Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

Store at 2-8 °C when not in use. This product is stable for at least one year when stored at 2-8 °C.

## Equipment and reagents required but not provided

| Catalog Number | Product Name |
|---|---|
| T2663 | 1 M Tris-HCl, pH 7.4 |
| T6066 | Trizma® Base |
| T5941 | Trizma® HCl |
| S5150 | 5 M Sodium Chloride |
| P9416 | TWEEN® 20 |
| B6429 | 10X Blocking Buffer |
| C0712 | CDP-Star Chemiluminescent Substrate |
| B1911 | BCIP®/NBT Liquid Substrate System (for colorimetric detection) |

## Preparation Instructions

1. 1 M Tris-HCl, pH 9.5: To 500 ml distilled deionized water, add 113.8 g Trizma Base and 9.46 g Trizma HCl. Stir until dissolved. If necessary, adjust pH to 9.5 with concentrated HCl or NaOH. Adjust final volume to 1 L with distilled deionized water.
2. Wash Buffer 1 (0.1 M Tris-HCl, pH 7.4, 0.15 M NaCl, 0.3% TWEEN 20): Prepare Wash Buffer 1 by combining 867 ml distilled deionized water, 100 ml of 1 M Tris-HCl, pH 7.4, 30 ml of 5 M NaCl, and 3 ml of TWEEN 20.
3. Wash Buffer 2 (0.1 M Tris-HCl, pH 9.5, 0.1 M NaCl): Prepare Wash Buffer 2 by combining 880 ml deionized water, 100 ml of 1 M Tris-HCl, pH 9.5 (see above) and 20 ml of 5 M NaCl.
4. 1X Blocking Buffer: Prepare by diluting 10X Blocking Buffer 1:10 with distilled deionized water.

## Procedure

The following procedure details the processing of a previously hybridized membrane following the completion of stringency washes.

A. Blocking the Membrane

1. Wash the membrane with Wash Buffer 1 for five minutes with gentle agitation to remove any residual SDS carried over from the hybridization. Use 1-1.5 ml/cm$^2$ of membrane. Transfer the membrane to a clean container.
2. Incubate the membrane with 1X Blocking Buffer for 60 minutes at room temperature with gentle agitation. Use approximately 1 ml/cm$^2$ of membrane.
3. Proceed to Section B, Incubation with Anti-FITC Alkaline Phosphatase . Alternatively, the blocked membrane can be stored overnight covered in 1X Blocking Buffer at 2-8 °C.

B. Incubation of the Membrane with Anti-FITC-Alkaline Phosphatase

1. Dilute the Anti-FITC-Alkaline Phosphatase in 1X Blocking Buffer. For colorimetric detection, dilute the conjugate 1:100 to 1:200. For chemiluminescent detection, dilute the conjugate 1:1000 to 1:2000.
2. Incubate the membrane with diluted conjugate for 30-60 minutes at room temperature with gentle agitation. Use 1 ml/cm$^2$ of membrane.

C. Washing the Membrane

1. Using forceps, transfer the membrane to a clean container containing Wash Buffer 1. Use 1-1.5 ml/cm$^2$ of membrane.
2. Wash the membrane for 5 minutes with gentle agitation.
3. Repeat steps 1 and 2 twice for a total of three washes with Wash Buffer 1.
4. Wash the membrane with Wash Buffer 2 for 5 minutes with gentle agitation. Use 1-1.5 ml/cm$^2$ of membrane.
5. Repeat step 4 for a total of two washes with Wash Buffer 2.
6. Continue to Section D1 for chemiluminescent detection or Section D2 for colorimetric detection.

D1. Detection of Labeled Probe with CDP-Star Chemiluminescent Substrate Solution

1. Using forceps, transfer the membrane to a clean, appropriately sized container.
2. Using aseptic technique, add CDP-*Star* to the membrane (50 µl/cm$^2$ membrane).
3. Incubate for 5 minutes at room temperature with gentle agitation to allow CDP-*Star* to fully cover the membrane.
4. Using forceps, remove the membrane and drain off any excess CDP-*Star* onto an absorbent material. Do not let the membrane dry out.
5. Transfer the membrane to a development folder, with the sample side up. Place into a light-tight film cassette.
6. Expose the membrane to film from 30 seconds to overnight at room temperature. Exposure time should be adjusted accordingly to achieve the highest signal-to-noise ratio.
7. Develop film as per manufacturer's instructions.

D2. Colorimetric Detection of Labeled Probe with BCIP/NBT Liquid Substrate System

1. Using forceps, transfer the membrane to a clean, appropriately sized container.
2. Using aseptic technique, add BCIP/NBT to the membrane (100-1000 µl/cm$^2$ membrane).
3. Develop for 5-60 minutes at room temperature with gentle agitation.
4. Wash the membrane with distilled deionized water for 5-15 minutes to quench development.
5. Dry membrane at 60-80 °C for at least 5 minutes.
6. Store membrane protected from direct light.

Related Products

| Catalog Number | Product Name |
|---|---|
| N1406, N3656, N3781, N3906 | BioBond™ Neutral Charge Nylon Membrane |
| N5281, N5406, N5531, N5656 | BioBond™ Plus Positively Charged Nylon Membrane |
| UALK | CDP-*Star*® Universal Alkaline Phosphatase Detection Kit |
| H7033 | PerfectHyb™ Plus Hybridization Buffer |



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