



# Anti-Biotin Alkaline Phosphatase

Order no. 130-092-612

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## 1. Description

|                       |  |
|-----------------------|--|
| <b>Clone</b>          | Bio3-18E7 (isotype: mouse IgG1)  |
| <b>Product format</b> | 1 mL Anti-Biotin Alkaline Phosphatase: monoclonal Anti-Biotin antibody conjugated to alkaline phosphatase.<br>The antibody is supplied in a solution containing stabilizer and 0.05% sodium azide. |
| <b>Product size</b>   | Up to 100 tests (100 µg in 1 mL).  |
| <b>Storage</b>        | Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.   |

### 1.1 Background and product applications

Anti-Biotin Alkaline Phosphatase has been developed as a secondary antibody for indirect labeling in conjunction with biotinylated primary antibodies. Alkaline phosphatase works with precipitating substrates such as NBT/BCIP as well as soluble substrates such as pNPP to produce a colorimetric reaction.

The anti-biotin antibody will also detect endogenous biotin or biotinylated molecules present in the cell, therefore it is important to include an endogenous biotin blocking step as well as the appropriate controls whenever possible.

### Product applications

- Indirect labeling of frozen sections or formalin-fixed, paraffin-embedded tissue sections previously stained with a biotinylated primary antibody.
- Detection of biotinylated proteins or free biotin by ELISA.

### 1.2 Recommended antibody dilution

- For immunohistochemistry and immunocytochemistry a working dilution of 1:100 is recommended. For other applications, such as ELISA, the optimal working dilution must be determined.

### 1.3 Reagent and material requirements

#### For immunohistochemical staining of paraffin-embedded tissue sections

- Silane-coated slides
- Coverslips
- Hellendahl jars
- Hydrophobic pen
- Formalin fixed, paraffin-embedded section-compatible biotinylated primary antibody
- Roti®-Histol (xylol substitute) (Carl Roth # 6640.1)
- Isopropanol
- Ethanol dilution series (96%, 80%, 70% (v/v))
- 10× Target Retrieval Solution, pH 6.0 (DakoCytomation # S1699)
- Biotin-Blocking System (DakoCytomation # X0590)
- 0.01 M Tris-buffered saline (TBS), pH 7.4
- Milk powder
- Washing buffer: TBS with 0.5% (w/v) milk powder
- Blocking buffer: TBS with 2.5% (w/v) milk powder
- Antibody dilution buffer: TBS with 1% (w/v) milk powder
- SIGMAFAST™ BCIP/NBT Tablets (Sigma # B5655)
- 0.1% Nuclear Fast Red in 5% (w/v) aluminum sulfate solution, filtered.
- Levamisole Solution
- Non-aqueous Mounting medium (Roti-Histokit, Carl Roth # 6638.1)
- Deionized water

#### For immunocytochemical staining

- Silane-coated Slides
- Hellendahl jars
- Hydrophobic pen
- Phosphate-buffered saline (PBS)
- Biotinylated primary antibody
- SIGMAFAST Fast Red TR/Naphtol AS-MX Tablets (Sigma # F4648)
- (Optional) Mayer's hemalum solution
- (Optional) Aqueous Mounting medium (Fluoromount-G, Southern Biotech # 0100-01)
- (Optional) Inside Stain Kit for intracellular staining (# 130-090-477)

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## 2. Protocols

### 2.1 Immunohistochemical staining of paraffin-embedded tissue sections

- Deparaffinize and rehydrate the tissue section by serial immersion in Hellendahl jars containing the following:
  - 2 × 5 min Roti-Histol
  - 2 × 3 min isopropanol
  - 2 × 3 min 96% ethanol
  - 2 × 3 min 80% ethanol
  - 2 × 3 min 70% ethanol
  - 2 × 3 min deionized water
- For antigen retrieval, prepare the Target Retrieval Solution according to the manufacturer's instructions and heat to 89 °C. Incubate tissue sections for 40 min, then allow to cool to room temperature.
- Wash slides 2 × 3 minutes with 0.01 M TBS in a Hellendahl jar.
- Dab slides dry around the section and encircle it with a hydrophobic pen.
- Block endogenous biotin by pipetting enough of Solution 1 of the Biotin-Blocking System to cover the tissue section. Incubate for 5 minutes at room temperature in a humidified chamber.
- Rinse 1 × with 0.01 M TBS. Cover the tissue section as before with Solution 2 of the Biotin-Blocking System. Incubate for 5 minutes at room temperature in a humidified chamber.
- Wash slide 2 × 3 minutes with 0.01 M TBS in a Hellendahl jar.
- Block non-specific binding of the primary antibody by incubating slides at room temperature for 20 min in blocking buffer.
- Drain off supernatant by tilting the slides. Do not wash slides.
- Pipette 100-150 µL of the biotinylated primary antibody, diluted according to the manufacturer's instructions in antibody dilution buffer, to each section and incubate slide for 1 hour at room temperature in a humidified chamber.
- Wash slide 3 × 3 minutes with washing buffer in a Hellendahl jar.
- Pipette 100-150 µL of the Anti-Biotin Alkaline Phosphatase antibody, diluted 1:100 in antibody dilution buffer, to each section and incubate slide for 45 minutes at room temperature in a humidified chamber.
- During the incubation, prepare the SIGMAFAST BCIP/NBT substrate according to the manufacturer's instructions. For every 1 mL of substrate solution, add 1 drop of Levamisole Solution.
- Wash slides 3 × 3 minutes with 0.01 M TBS in a Hellendahl jar.
- Pipette enough substrate solution to cover the tissue section and incubate slide for 20 minutes at room temperature in a humidified chamber.
- Wash slides 3 × 2 minutes with deionized water in a Hellendahl jar.

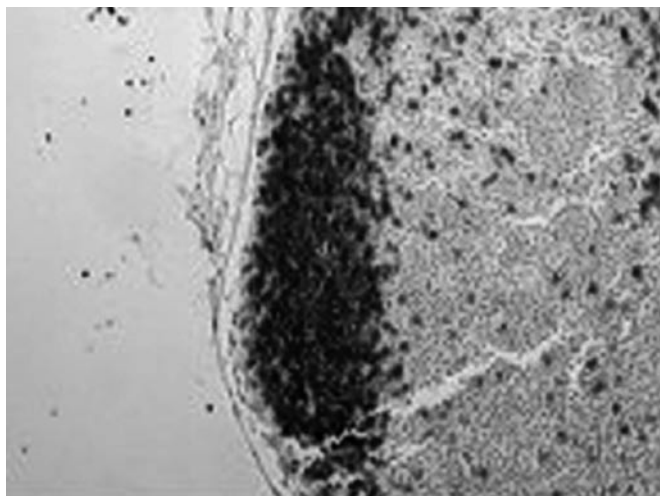
- Counterstain tissue sections by dipping sections for no longer than 45 seconds in Nuclear Fast Red solution.
- Wash slides thoroughly with tap water followed by deionized water.
- Dehydrate the tissue sections by serial immersion in Hellendahl jars containing the following:
  - 2 × 3 min 70% ethanol
  - 2 × 3 min 80% ethanol
  - 2 × 3 min 96% ethanol
  - 2 × 3 min 96% isopropanol
  - 2 × 3 min Roti-Histol
- Apply mounting medium and a coverslip to each slide.

### 2.2 Immunocytochemical staining

- Cytospin a maximum of  $1 \times 10^5$  cells per Silane-coated slide.
- Allow cells to dry for 30 minutes at 37 °C.
- Encircle cells with a hydrophobic pen.
- Incubate slides for 2 minutes with PBS in a Hellendahl jar.
- (Optional) For Intracellular staining, apply 300 µL of Inside Fix reagent and incubate for 10 minutes at room temperature. Wash slides 3 × 5 minutes with fresh PBS in a Hellendahl jar.
- Pipette 250 µL of the biotinylated primary antibody, diluted according to the manufacturer's instructions in PBS, to each slide and incubate for 30–45 minutes at room temperature in a humidified chamber.
  - ▲ **Note:** (Optional) For intracellular staining, the biotinylated primary antibody should be diluted in Inside Perm reagent.
- Wash slides 3 × 5 minutes with fresh PBS in a Hellendahl jar.
- Pipette 250 µL of the Anti-Biotin Alkaline Phosphatase antibody, diluted 1:100 in PBS, to each section and incubate slide for 45 minutes at room temperature in a humidified chamber.
  - ▲ **Note:** (Optional) For intracellular staining, the Anti-Biotin Alkaline Phosphatase antibody should be diluted in Inside Perm reagent.
- During the incubation, prepare the SIGMAFAST Fast Red TR/Naphtol AS-MX substrate according to the manufacturer's instructions.
- Wash slides 3 × 5 minutes with fresh PBS in a Hellendahl jar.
- Pipette 250 µL of the substrate solution to each slide and incubate for 15 minutes at room temperature in a humidified chamber.
- Wash slides 2 × 5 minutes with deionized water in a Hellendahl jar and then allow to dry.
- (Optional) Counterstain slides by immersion for 1 minute in filtered Meyer's hemalum solution in a Hellendahl jar.
- Wash slides for 2 minutes with deionized water in a Hellendahl jar.
- Apply aqueous mounting medium and a coverslip to each slide.

### 3. Example of immunohistochemical staining using the Anti-Biotin Alkaline Phosphatase antibody

Methacarn-fixed, paraffin-embedded serial sections of a rat lymph node were stained with a biotin-conjugated CD45R primary antibody followed by Anti-Biotin Alkaline Phosphatase. Color development was achieved using SIGMAFAST BCIP/NBT as a substrate.



#### Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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