UltraBrite™ Green IHC chromogen (AP)

CATALOG NO: M1309-30
AMOUNT: 30 ml
FORM: Liquid
STORAGE CONDITIONS: +4°C. Do not freeze.
SHELF LIFE: Stable up to 12 months. Chromogen is light sensitive, store away from light.
APPLICATIONS: IHC, ISH (in situ hybridization)

Background: The UltraBrite™ green IHC chromogen (AP) is a substrate-chromogen system designed to be used for either immunohistochemistry (IHC) or ISH when utilizing alkaline phosphatase. UltraBrite™ green IHC chromogen (AP) has been modified to increase stability and staining intensity, producing a strong green color that is insoluble in alcohol and xylene substitutes (both aliphatic hydrocarbon and citrus based); therefore sections can be dehydrated in alcohol, cleared in xylene substitute*, and permanently mounted. However, we recommend air drying slides and then permanently mounting. This chromogen substrate system may be used for both automation and manual use.

Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>M1309-30 Amount</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>UltraBrite™ green AP Substrate</td>
<td>30 ml</td>
<td>M1309-30-1</td>
</tr>
<tr>
<td>UltraBrite™ green AP Chromogen</td>
<td>2.4 ml</td>
<td>M1309-30-2</td>
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Cervical tissue incubated with a primary antibody to Ki-67, stained with anti-Mouse Reagent and UltraBrite™ Green AP Chromogen/Substrate

UltraBrite™ Green IHC Chromogen (AP) Protocol:

A. Working Solution:
Per 920 µl of UltraBrite™ green AP Substrate Reagent, add 80 µl of UltraBrite™ green AP chromogen to make 1 ml. Replace tip, mix, and allow solution to reach room temperature before using. Mix well, and keep protected from bright light. At room temperature, this solution is stable for up to 6 hours. When refrigerated and protected from light, this solution is stable for up to 1 day.

Note: The UltraBrite™ green IHC chromogen (AP) chromogen substrate working solution is light sensitive and should be kept away from light as much as possible. Working solution should be made fresh.

Additional Materials Required (Not Provided):
1. Xylene substitute for clearing prior to mount (either Shandon Xylene Substitute, ThermoFisher Scientific Cat. No. 9990505 or Citrus Clearing Solvent, ThermoFisher Scientific Cat. No. 8301)
2. Distilled or deionized water (dH2O)
3. 70% and 100% Ethanol (Reagent Grade)

B. Protocol/Staining Procedure:
1. After adding secondary antibody, add UltraBrite™ green AP Chromogen/Substrate to cover the tissue section (~150 µL) and allow color to develop for 10 - 15 minutes at room temperature.
2. Tap off excess chromogen. Wash the slides with dH2O for two minutes. Tap off excess dH2O.
3. Counterstain with Hematoxylin or Nuclear Fast Red for good contrast. Wash with dH2O.

If desired, dehydrate the slides in 70% alcohol for 1 minute followed by 100% alcohol for 1 minute. Then, clear sections in xylene substitute for 1 minute and then mount using permanent histological mounting medium.

Caution: Using xylene instead of xylene substitute will cause the signal to dissipate. Ensure you are using xylene substitute. See ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED section for recommended commercially available xylene substitutes.

Related Products:
- UltraBrite™ Red IHC chromogen (AP) (Cat. No. M1305-30)
- UltraBrite™ Red IHC chromogen (AP Plus) (Cat. No. M1306-30)
- UltraBrite™ Blue IHC chromogen (AP) (Cat. No. M1307-30)
- UltraBrite™ Blue IHC chromogen (HRP) (Cat. No. M1308-30)
- UltraBrite™ Yellow IHC Chromogen (HRP) (Cat. No. M1310-30)
- UltraBrite™ Black IHC chromogen (HRP) (Cat. No. M1311-30)
- UltraBrite™ Red IHC chromogen (HRP) (Cat. No. M1312-30)

For Research Use Only! Not to be used in humans.