

## UltraBrite™ Blue IHC chromogen (AP)

**CATALOG NO:** M1307-30  
**AMOUNT:** 30 ml  
**FORM:** Liquid  
**STORAGE CONDITIONS:** +4°C  
**SHELF LIFE:** Stable up to 12 months. Chromogen is light sensitive, store away from light.  
**APPLICATIONS:** IHC, ISH (in situ hybridization)

**BACKGROUND:** The UltraBrite™ blue IHC chromogen (AP) is a substrate-chromogen system designed to be used for either immunohistochemistry (IHC) or ISH when utilizing alkaline phosphatase. UltraBrite™ blue IHC chromogen (AP) has been formulated for increased stability of the working solution while producing a distinct bright blue color. It 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:

Components	M1307-30	Part Number
UltraBrite™ blue IHC chromogen (AP) System buffer	30ml	M1307-30-1
UltraBrite™ blue IHC chromogen (AP) Chromogen	1ml	M1307-30-2
Empty Mixing Bottle	1	M1307-30-3

### UltraBrite™ BLUE IHC CROMOGEN (AP) PROTOCOL:

#### A. WORKING SOLUTION:

Aliquot 1 mL of UltraBrite™ blue IHC chromogen (AP) Substrate Buffer in a mixing bottle. Add one drop (~20 µL) of concentrated UltraBrite™ blue IHC chromogen (AP) chromogen solution. Replace tip, mix, and allow solution to reach room temperature before using.

**Note:** The UltraBrite™ blue IHC chromogen (AP) chromogen substrate working solution is light sensitive and should be kept away from light as much as possible. Working solution is stable for up to 6 hours in the dark; any solution not used during this period should be discarded. For optimal staining, use freshly made solution.

#### B. PROTOCOL/STAINING PROCEDURE:

Following alkaline phosphatase incubation, wash tissue sections with wash buffer, then follow protocol of choice:

Protocol	Staining Procedure	Incubation Times
Pre-Mix Working Solution: (Automation)	UltraBrite™ blue IHC chromogen (AP) working solution has a 6-hour stability and can be loaded directly onto instrument as a single solution. Reduce exposure to light to achieve optimal staining. Working solution is applied directly to slide.	Working Solution: 10 - 20 min
On Board Mixing (Automation)	Instruments that have on-board mixing capability can load the chromogen and substrate-buffer components independently. Working solution is made mixing reagents 1:50 using on-board mixing station before application to slide.	Working Solution: 10 - 20 min
Manual Use	Mix substrate-chromogen and buffer in a 1:50 ratio and apply directly to slide.	Working Solution: 10 - 20 min

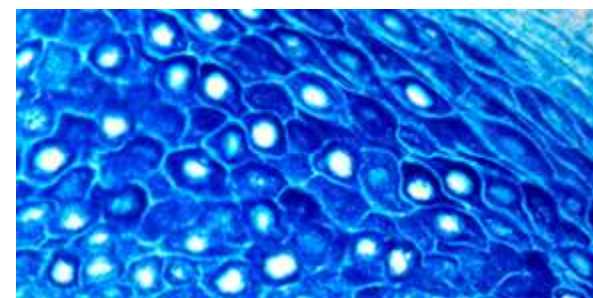
#### C. COUNTERSTAIN:

Counterstain with Hematoxylin or Nuclear Fast Red for good contrast. Wash with distilled or de-ionized H<sub>2</sub>O followed by immuno wash buffer.

#### D. MOUNTING:

We recommend air drying slides (instead of dehydrating or clearing in alcohol and xylene-substitute). After rinsing off counterstain in distilled or de-ionized H<sub>2</sub>O, leave slides on benchtop for at least 20 minutes to air dry, then permanently mount. Alternatively, you may dehydrate sections in increasing concentrations of ethanol up to 100%, clear in a xylene substitute\*, and mount with a permanent mounting medium.

\*Note: Use xylene-substitute instead of xylene.



**Fig: Immunohistochemical analysis:** Formalin fixed paraffin embedded human tonsil stained with a high molecular weight CK antibody labeled with UltraBrite™ blue IHC chromogen (AP) produces a distinct bright blue color.



Formalin-fixed paraffin-embedded human tonsil stained with pre-diluted mouse CD68 antibody labeled with UltraBrite™ chromogen substrate (DAB, HC), pre-diluted rabbit lambda light-chain antibody labeled with UltraBrite™ IHC chromogen (AP), pre-diluted rabbit kappa light-chain antibody labeled with UltraBrite™ red IHC chromogen (AP, plus), high molecular weight cytokeratin labeled with UltraBrite™ yellow IHC chromogen (HRP), and methyl green nuclear counterstain.

#### 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 (HRP) (Cat. No. M1308-30)
- UltraBrite™ Green IHC chromogen (AP) (Cat. No. M1309-30)
- 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.***