

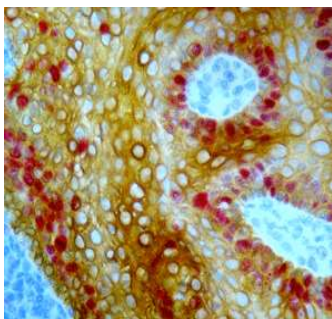
## UltraBrite™ Red IHC chromogen (AP)

**CATALOG NO:** M1305-30  
**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™ red IHC chromogen (AP) is a substrate-chromogen system designed to be used for either IHC or ISH when using alkaline phosphatase detection. The system produces a brilliant dark red color and is insoluble in organic solvents; therefore, sections can be dehydrated in alcohol, cleared in xylene (or a xylene-substitute), and permanently mounted. This chromogen substrate system may be used for both automation and manual use.

### KIT CONTENTS:

Components	M1305-30	Part Number
UltraBrite™ Red IHC chromogen (AP) System buffer	30ml	M1305-30-1
UltraBrite™ Red IHC chromogen (AP) Chromogen	1ml	M1305-30-2
Empty Mixing Bottle	1	M1305-30-3



**Fig: Immunohistochemical analysis:** Formalin fixed paraffin embedded human tonsil double stained using mouse-HRP/rabbit-AP IHC kit. Primary rabbit anti-Ki67 antibody was visualized with UltraBrite™ red IHC chromogen (AP), while primary mouse anti-HMW CK antibody was visualized with UltraBrite™ yellow IHC chromogen (HRP), producing a distinct golden yellow color contrasting with the bright red.

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

#### A. WORKING SOLUTION:

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

**Note:** The working chromogen-substrate solution should be prepared fresh and used within 20-30 minutes of preparation. Any solution not used during this period should be discarded.

#### B. PROTOCOL/STAINING PROCEDURE:

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

Protocol	Staining Procedure	Incubation Times
Batch Mode (Automation)	Using Batch Mode on your instrument, wait for machine to notify you when ready, then mix chromogen and buffer in a 1:50 ratio and load onto instrument. Working solution is stable for only 20-30 minutes and should be applied to slide immediately for best results.	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:

Use Hematoxylin for good contrast. Wash with distilled or deionized H<sub>2</sub>O followed by immuno wash buffer.

#### D. MOUNTING:

Sections can be dehydrated in alcohol, cleared in xylene or xylene substitute and permanently mounted. Alternatively, slides can be air dried (instead of alcohol and xylene). 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 or use aqueous mounting media.

#### RELATED PRODUCTS:

- 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™ 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.**