

UltraBrite™ Red IHC chromogen (AP Plus)

CATALOG NO: M1306-30
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: Substrate/chromogen system for use with alkaline phosphatase (AP) detection in immunohistochemistry (IHC) or in situ hybridization (ISH) run on automated staining instruments as well as manual use.

BACKGROUND: Traditional formulations for red alkaline phosphatase chromogens are unstable upon mixing. This can be problematic for use in automated slide stainers. UltraBrite™ red IHC chromogen (AP Plus) has been formulated for on-slide and on-board mixing for any autostainer. Both components are stable and can be loaded at the beginning of the staining run, for walk away convenience. UltraBrite™ red IHC chromogen (AP Plus) produces a red color, ranging from pink to brilliant dark red. It 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	M1306-30	Part Number
UltraBrite™ Red IHC chromogen (AP Plus) Substrate buffer	30ml	M1306-30-1
UltraBrite™ Red IHC chromogen (AP Plus) Chromogen	30ml	M1306-30-2

UltraBrite™ RED IHC CROMOGEN (AP PLUS) PROTOCOL:

A. REAGENT PREPERATION FOR MANUAL USE:

UltraBrite™ red IHC chromogen (AP, plus) 1X Working Solution: In a clean dark bottle, mix equal volumes of UltraBrite™ red IHC chromogen (AP, plus) Buffer and UltraBrite™ red IHC chromogen (AP, plus) Chromogen in a 1:1 ratio.

MAKE FRESH: WORKING SOLUTION IS STABLE FOR ONLY 20-30 MINUTES.

B. REAGENT PREPARATION FOR AUTOMATION USE:

There is no preparation required for automation use. Reagents can be loaded directly on the instrument for on-slide mixing or onboard mixing.

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

Protocol	Staining Procedure	Incubation Times
On-Slide Mixing (Automation)	UltraBrite™ red IHC chromogen (AP, plus) has been formulated for direct application of the component solutions onto the slide in a specified order: sequential application of substrate buffer first, followed by chromogen in equal volumes with no washing or reagent "blow off" in between. Reversing the order of reagent application will increase background and yield suboptimal results.	Substrate Buffer: 1-5 min Chromogen: 10-20 min
Batch Mode (Automation)	Using Batch Mode on your instrument, wait for machine to notify you when ready, then mix chromogen and substrate-buffer in a 1:1 ratio and load onto instrument.	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 1:1 in on-board mixing station before application to slide.	Working Solution: 10 - 20 min
Manual Use	Mix chromogen and substrate-buffer in a 1:1 ratio and apply directly to slide. Working solution is stable for only 20-30 minutes and should be applied immediately for best results.	Working Solution: 10 - 20 min

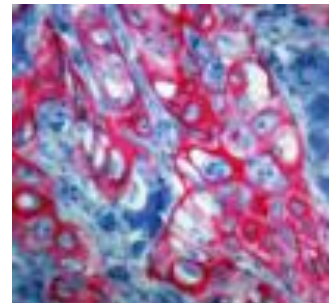
C. COUNTERSTAIN:

Counterstain with Hematoxylin for good contrast. Wash with distilled or de-ionized H₂O followed by immuno wash buffer.

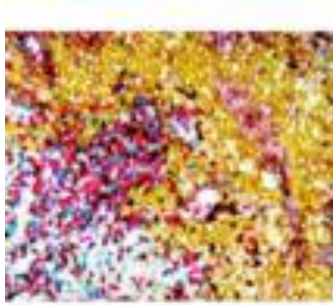
Sections can be dehydrated in alcohol, cleared in xylene or xylene substitute and permanently mounted.

D. MOUNTING:

Alternatively, slides can be air dried (instead of alcohol and xylene). After rinsing off counterstain in distilled or de-ionized H₂O, leave slides on benchtop for at least 20 minutes to air dry, then permanently mount or use aqueous mounting media.



Immunohistochemistry analysis: Formalin fixed paraffin embedded squamous cell carcinoma human tissue stained with a CK 5/6 antibody labeled with UltraBrite™ red IHC chromogen (AP, plus) produces a brilliant red color



Formalin-fixed paraffin-embedded human tonsil stained with pre-diluted mouse CD68 antibody labeled with UltraBrite™ IHC chromogen substrate (DAB, HC), pre-diluted rabbit lambda light-chain antibody labeled with UltraBrite™ blue IHC chromogen (AP, blue), 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™ 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.