PRODUCT INFORMATION & MANUAL

BLUE AP Plus Staining Kit (Colorimetric)

NBP3-12171

For research use only.
Not for diagnostic or therapeutic procedures.

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BLUE AP Plus

NBP3-12171

Effective Date: 3/7/2017

Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

BLUE AP Plus is a substrate-chromogen system designed to be used for either IHC or ISH when utilizing alkaline phosphatase. BLUE AP Plus has been reformulated to increase stability of the working solution while producing a distinct bright blue color. BLUE AP Plus is insoluble in alcohol and xylene substitutes; therefore sections can be dehydrated in alcohol, cleared in xylene substitute, and permanently mounted.

Principles of the Procedures

Substrate/chromogen in conjunction with alkaline phosphatase (AP)-based immunostaining or in situ hybridization systems.

Reagents Provided

Kit Contents	30 mL	110 mL
BLUE AP Plus Substrate Buffer	30 mL	110 mL
BLUE AP Plus Chromogen	1 mL	3 mL
Empty Mixing Bottle	1	1

Prepare the Following Solutions Before Use

1. Aliquot 1mL of BLUE AP Plus Substrate Buffer in a mixingbottle.
2. Add one drop (~20µl) of concentrated BLUE AP PlusChromogen solution.
3. Replace tip, mix, and allow solution to reach room temperature before using.

Note: The BLUE AP Plus 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.

Materials Required But Not Provided

Some of the reagents and materials required for IHC are not provided.

Storage and Handling

Store at 2°-8°C away from light. Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Staining Procedure

1. Once sections have been incubated with alkaline phosphatase, wash sections with wash buffer then follow protocol of choice:
   a) **Pre-Mix Working Solution: (Automation)** BLUE AP Plus working solution has 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. Incubate for 10 - 20 min
   b) **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 in on-board mixing station before application to slide. Incubate for 10 - 20 min.
   c) **Manual Use**: Mix substrate-chromogen and buffer in a 1:50 ratio and apply directly to slide. Incubate 10 - 20 min.
2. Counterstain with Hematoxylin or Nuclear Fast Red for good contrast. Wash with DI H2O followed by immuno wash buffer.
3. We recommend air drying slides (instead of dehydrating or clearing in alcohol and xylene-substitute). After rinsing off counterstain in DI H2O, leave slides on benchtop for at least 20 minutes to air dry, and then permanently mount.
4. 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.
   ***Notes:** Use xylene-substitute instead of xylene.

Recommendation:

For best color preservation and long term slide storage, we recommend to use Tissue Preservation Solution - HRP/AP assays (**NBP3-12178**) after counterstaining.

Precautions

1. Consult local and/or state authorities with regard to recommended method of disposal.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
4. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
5. If reagent contacts these areas, rinse with copious amounts of water.
6. Do not ingest or inhale any reagents.