PRODUCT INFORMATION & MANUAL

GREEN AP Plus Staining Kit (Colorimetric)

NBP3-12170

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

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Novus kits are guaranteed for 6 months from date of receipt

GREEN AP Plus

NBP3-12170

Effective Date: 3/7/2017

Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

GREEN AP Plus is a substrate-chromogen system designed to be used for either IHC or ISH when utilizing alkaline phosphatase. GREEN AP Plus 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.

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
GREEN AP Plus Substrate Buffer	30 mL	110 mL
GREEN AP Plus Chromogen	1 mL	3 mL
Empty Mixing Bottle	1	1

Prepare the Following Solutions Before Use

1. Aliquot 1mL of GREEN AP Plus Substrate Buffer in a mixing bottle.
2. Add one drop (~20µl) of concentrated GREEN AP Plus Chromogen solution.
3. Replace tip, mix, and allow solution to reach room temperature before using.
4. GREEN AP Plus chromogen-substrate working solution is light sensitive and should be kept away from light as much as possible.
5. Working solution should be prepared fresh in the dark.

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

6. Once sections have been incubated with alkaline phosphatase incubation, wash tissue sections with wash buffer, then follow protocol of choice:
   a. **Pre-Mix Working Solution: (Automation)** GREEN AP Plus working solution has to be prepared fresh 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 for 10-20 min.
7. Counterstain with Hematoxylin or other counterstain. Wash with DI water followed by immuno wash buffer.
8. Mounting: Slides should be air dried (do not dehydrate in alcohol or xylene). After rinsing off counterstain in DI H2O, leave slides on benchtop for at least 20 minutes to air dry, and then permanently mount.

Note: GREEN AP Plus chromogen-substrate working solution is light sensitive and should be kept away from light as much as possible. Working solution should be prepared fresh in the dark.

Recommendation:

For best color preservation and long term slide storage, we recommend using 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.