

Ultra High Def™ Blue-AP

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CRM-002-110

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Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

Ultra High Def™ Blue-AP is a substrate-chromogen system designed to be used for either IHC or ISH when utilizing alkaline phosphatase. Ultra High Def™ Green-AP has been reformulated to increase stability of the working solution while producing a distinct bright blue color. Ultra High Def™ Blue-AP 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
UHD™ Blue AP Substrate Buffer	30 mL	110 mL
UHD™ Blue AP Chromogen	1 mL	3 mL
Empty Mixing Bottle	1	1

Prepare the Following Solutions Before Use

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

Materials Required But Not Provided

All the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control slides, control reagents and other ancillary reagents are available from StatLab. Please refer to our website at: www.statlab.com

Storage and Handling

Store at 2-8°C. Do not use product after the expiration date printed on vial. If reagents are stored under a condition other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly.

Staining Procedure

1. Once sections have been incubated with alkaline phosphate, wash sections with wash buffer, then follow protocol of choice:
 - a. **Pre-Mix Working Solution: (Automation)** Ultra High Def™ Blue- 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. Incubate for 10-20 minutes.
 - 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 minutes.
 - c. **Manual Use:** Mix substrate-chromogen and buffer in a 1:50 ratio and apply directly to slide. Incubate for 10 – 20 minutes.
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 substitutes). 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 alcohol up to 100%, clear in a xylene-substitute, and mount with a permanent mounting media.

Note: Use xylene substitute instead of xylene

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

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact StatLab Headquarters: 2090 Commerce Drive, McKinney, TX 75069. Email our team at ihctech@statlab.com or Call at (800) 442-3573.

