Ultra High Def™ DAB

ACR-021

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Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

Ultra High Def™ DAB is a widely used chromogen for immunoperoxidase staining, and is well accepted among pathologists because of its increased sensitivity and ability to give cleaner background as compared to amino ethylcarbazole (AEC). Specimens stained in DAB can be dehydrated, cleared, and mounted for permanent record keeping. DAB is more sensitive and stable than traditional working DAB substrates.

Principles of the Procedures

Substrate/chromogen in conjunction with peroxidase-based immunostaining systems. Ultra High Def™ DAB offers several noteworthy improvements and benefits as compared with traditional working DAB solutions. Stable DAB/Plus is much more sensitive, providing the cost-effective option of diluting the primary antibody. Being stable for 24 hours (as opposed to 6 hours for traditional DAB working solutions), Ultra High Def™ DAB allows the user more convenience. Hazardous waste generation from spent DAB solution is also significantly reduced. Ultra High Def™ is ideal for high volume labs and automated stainers.

Peroxidase from the antibody detection system reacts with H_2O_2 substrate to degrade it, which then reacts with DAB, precipitating it at positive sites yielding a dark brown color.

Reagents Provided

Kit Contents	Volume
Concentrated Amber-Colored DAB Chromogen	5 mL
Clear Stable DAB Substrate/Buffer	200 mL
Empty Mixing Dropper Bottle	1

Prepare the Following Solutions Before Use

1. Aliquot 1mL of Ultra High Def™ DAB Substrate Buffer in a mixing bottle.
2. Add one drop (~20µl) of concentrated Ultra High Def™ DAB Chromogen solution.
3. Replace tip, mix, and allow solution to reach room temperature before using.
4. Store at 2-8° C when not in use.
5. The working chromogen-substrate solution is stable for up to 24 hours, for optimal results prepare fresh reagent. Any solution not used after this period should be discarded.

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. Away from light. 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 horseradish peroxidase, wash sections with wash buffer, then follow protocol of choice:
   a. **Pre-Mix Working Solution: (Automation)** Ultra High Def™ Black HRP has a working solution stability of at least 1 day 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 5-10 minutes.
   b. **On Board Mixing: (Automation)** Instruments that have on-board mixing capability can load the chromogen and substrate buffer components independently. Working solutions is made mixing reagents 1:50 (1 drop to 1 mL) in on board mixing station before application to slide. Incubate for 5-10 minutes
   c. **Manual Use:** Mix substrate-chromogen and buffer in a 1:50 ratio and apply directly to slide. Incubate for 5-10 minutes.
2. Counterstain with Hematoxylin or other counterstain.
3. Wash with DI H_2O followed by Immuno wash buffer.
4. Slides can be dehydrated through alcohol and xylene or xylene substitute or can be air dried, then permanently mounted.

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/result 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. Call at (800) 442-3573 or email our team at ihctech@statlab.com