

## High Contrast DAB (Liquid Format)

K 055

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### Intended Use

For In Vitro Diagnostic Use

### Summary and Explanation

3,3'-Diaminobenzidine (DAB), is a widely used chromogen for immunoperoxidase staining, and is well accepted among pathologists because of its increased sensitivity and decreased background when compared to amino ethylcarbazole (AEC). Specimens stained with DAB can be dehydrated, cleared, and permanently mounted. High Contrast DAB is more sensitive and stable than traditional working DAB solutions. High Contrast DAB offers several noteworthy improvements and benefits as compared to traditional working DAB solutions.

High Contrast DAB is highly sensitive, providing the cost-effective option of further diluting the primary antibody. Being stable for 2 weeks (as opposed to 6 hours for traditional DAB working solutions), High Contrast DAB allows the user the convenience of making one working solution for the entire week's work. Hazardous waste generation from unused DAB solution is also significantly reduced. High Contrast DAB is ideal for high volume labs and automated stainers. High Contrast DAB stains dark chocolate brown and is easy to distinguish between other chromogens such as our PermaRed and Blue series when multiple chromogens are used on a single slide.

### Principles of the Procedures

Substrate/chromogen in conjunction with peroxidase-based immunostaining systems. In the presence of hydrogen peroxide, DAB is converted to an insoluble brown reaction product and water by the enzyme horseradish peroxidase (HRP).

### Reagents Provided

Kits Contents	Volume
Concentrated Dark Purple-Colored High Contrast DAB Chromogen	5 mL
Clear High Contrast DAB Substrate Buffer	200 mL
Empty Mixing Bottle	1

### Prepare the Following Solutions Before Use

1. Aliquot 1mL of High Contrast DAB Buffer in mixing bottle.
2. Add 20µL (one drop) of concentrated High Contrast DAB Chromogen. Replace tip and mix.
3. The working High Contrast DAB solution is stable for at least 1 week and should be prepared in an opaque bottle.
4. For better results use freshly prepared working solution.

### Materials Required But Not Provided

Some of the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control reagents and other ancillary reagents are available from Diagnostic BioSystems. Please refer to the Diagnostic BioSystems website at [www.dbiosys.com](http://www.dbiosys.com).

### Storage and Handling

Store at 2-8°C. Reagents and working solution are light sensitive; protect from exposure to light. Store reagents and working solution in opaque bottle. Do not use beyond the expiration dates stated on the labels.

### Staining Procedure

1. After peroxidase incubation, wash tissue sections with wash buffer.
2. Wipe slides removing excess buffer. Add enough drops of working High Contrast DAB Solution to cover tissue sections.
3. Incubate for 5-10 minutes at room temperature. For optimal results, observe reaction under the microscope for signal development. Once the desired signal to noise ratio is achieved, stop the reaction by washing slides in buffer.

### Precautions

1. DAB, a suspected carcinogen, can cause skin irritation upon contact.
2. Avoid contact with clothes and exposed skin.
3. If contacted, flush immediately with tap water.
4. Consult local and/or state authorities with regard to recommended method of disposal.
5. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
6. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
7. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
8. If reagent contacts these areas, rinse with copious amounts of water.
9. 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 Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or [techsupport@dbiosys.com](mailto:techsupport@dbiosys.com).

