



# Product Manual

## **HIGHDEF<sup>®</sup> DAB Chromogen/Substrate Set**

Catalog #: ENZ-ACC105-0200

### **Liquid Format**

**NOTE:** This version contains a change to the instructions for preparation of the working solution.

For the latest product information, including support documentation, visit us online:

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Carefully note the handling and storage conditions of each kit component.



Please read entire booklet before proceeding with the assay.



Please contact Enzo Life Sciences Technical Support if necessary.

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## INTENDED USE

Substrate/chromogen set in conjunction with peroxidase-based immunostaining systems.

## INTRODUCTION

DAB, a widely used chromogen for immunoperoxidase staining, is well accepted among pathologists because of its increased sensitivity and ability to give cleaner background as compared to amino ethyl carbazole (AEC). Specimens stained with DAB can be dehydrated, cleared, and mounted for permanent record keeping. HIGHDEF<sup>®</sup> DAB Chromogen/Substrate Set is more sensitive and stable than traditional working DAB solutions.

## PRODUCT BENEFITS

HIGHDEF<sup>®</sup> DAB Chromogen/Substrate Set offers several noteworthy improvements and benefits as compared with traditional working DAB solutions:

1. HIGHDEF<sup>®</sup> DAB Chromogen/Substrate Set is much more sensitive, providing the cost-effective option of diluting the primary antibody.
2. Being stable for 2 weeks at 4°C (as opposed to 6 hours for traditional DAB working solutions), HIGHDEF<sup>®</sup> DAB Chromogen/Substrate Set allows the user the convenience of making one working solution for the entire work week. For best results, make working solutions as you need them.
3. Hazardous waste generation from spent DAB solution is also significantly reduced.
4. HIGHDEF<sup>®</sup> DAB Chromogen/Substrate Set is ideal for high volume labs and automated stainers.



## PRINCIPLE

Peroxidase from the antibody detection system reacts with H<sub>2</sub>O<sub>2</sub> substrate to degrade it, which then reacts with DAB, precipitating it at positive sites yielding a dark brown color.

## SET CONTENTS

1. 8 mL (25X) concentrated amber-colored HIGHDEF<sup>®</sup> DAB Chromogen.
2. 200 mL clear HIGHDEF<sup>®</sup> DAB Substrate Buffer.

## STORAGE

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. Stable for one year upon receipt.

## WORKING SOLUTION

To make the working solution, aliquot 1 mL of HIGHDEF<sup>®</sup> DAB Substrate Buffer into a mixing bottle. Add 40 µL of concentrated HIGHDEF<sup>®</sup> DAB Chromogen. Replace tip and mix well by inverting the bottle.

**Note:** The working solution is stable for at least 2 weeks at 4°C and for up to 6 hours at room temperature and should be prepared in an opaque bottle. Store at 2-8°C when not in use. Any solution not used after this period should be discarded.



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## PROCEDURE

1. Immediately after peroxidase incubation, wash tissue sections with wash buffer. To avoid background, ensure that the tissue remains wet throughout the process.
2. Wipe slides removing excess buffer. Add enough drops of working 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 PBS.

Note: If you premixed DAB and substrate buffer, you may notice precipitation of DAB. Mix well by inverting before you use it and store at 4°C.

## PRECAUTIONS

DAB, a suspected carcinogen, can cause skin irritation upon contact. Avoid contact with clothes and exposed skin. If contacted, flush immediately with tap water. Follow instructions provided by local authorities for disposal.



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**NOTES**



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