



For In Vitro Diagnostic Use

IMMUNODETECTOR DAB SUBSTRATE-CHROMOGEN KIT

Intended Use *For in vitro diagnostic use*

Summary And Explanation ImmunoDetector DAB Substrate-Chromogen kit is suitable for use in HRP Detection systems and allow for the demonstration of tissue antigens or nucleic acids in paraffin-embedded tissues, cryostat sections, cytosmears, and cell preparations. The substrate chromogen is the final step in the detection portion; it enables the antibody antigen complex or nucleic acid-probe complex to be viewed under the light microscope. This occurs because DAB acts as an electron donor in the presence of the enzyme horseradish peroxidase; DAB gets oxidized and produces a brown color at the site of the target antigen or nucleic acid.

Presentation DAB (3'3'diaminobenzidine) is a chromogen (color forming molecule) that develops into a brown precipitate. DAB forms a permanent record of the results of the stain when coverslipped with an organic based resin, such as Perma Mounter (BSB 0094-BSB 0097).

Availability

Catalog No.	Buffer-Substrate	Chromogen
BSB 0015	15 mL	1 mL
BSB 0016	50 mL	3 mL
BSB 0017	100 mL	6 mL
BSB 0018	200 mL	12 mL
BSB 0018A	500 mL	25 mL
BSB 0018B	1000 mL	60 mL
BSB 0019A	-	100 mL
BSB 0019B	-	60 mL
BSB 0019C	-	12 mL
BSB 0019G	-	6 mL
BSB 0019	1000 mL	-
BSB 0019D	500 mL	-
BSB 0019E	200 mL	-
BSB 0019F	100 mL	-

Storage

Store at 2-8°C

Stability 3 years

The ImmunoDetector DAB Buffer-Substrate and ImmunoDetector DAB Chromogen solutions are stable for up to 3 years from when originally produced (see expiration date on product label). These products are stable up to the expiration date on the label. Do not use this product after the expiration date. Adhere to all local laws when disposing of this product.

Protocol

Suggested protocol on reverse

Preparation of Working Solutions

To prepare a working ImmunoDetector DAB Substrate-Chromogen solution, first shake the **DAB Chromogen** solution, then add and mix 1 drop of **Chromogen** to 1 mL of **DAB Buffer-Substrate** per 1 mL of DAB Substrate-Chromogen required.

Working DAB Substrate-Chromogen Required	1 mL	2 mL	3 mL
DAB Buffer	1 mL	2 mL	3 mL
DAB Chromogen	1 drop	2 drops	3 drops

Recommended Protocol

- Pretreatment**
- Cut and mount 3-4 micron formalin-fixed paraffin-embedded tissues on positive charged slides.
 - Air dry for 2 hours at 58° C.
 - Deparaffinize, dehydrate and rehydrate tissues.
 - Subject tissues to heat epitope retrieval using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
 - Any of three heating methods may be used:
 - Electric Pressure Cooker
Place standoff rack at base of pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high, and incubate for 15 minutes. Open and immediately transfer slides to room temperature in ImmunoDNA Retriever solution.
 - Water Bath Method
Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a water bath set at 95°-99° C. Incubate for 30-60 minutes.
 - Conventional Steamer Method
Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a Steamer, cover and steam for 30-60 minutes.
 - After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
 - Wash slides with IHC wash buffer or DI water.
 - Continue IHC staining protocol.

Immunohistochemical Protocol

Step	ImmunoDetector HRP	PolyDetector HRP
Peroxidase Blocker	5 minutes	5 minutes
Primary Antibody	30 minutes	45 minutes
Secondary Biotinylated Link	10 minutes	N/A
HRP Label	10 minutes	45 minutes
ImmunoDetector DAB Substrate Chromogen	10 minutes	10 minutes
Counterstaining	Time varies with counterstain	Time varies with counterstain

Precautions

For professional users. Minimize microbial contamination of reagents. Proper handling procedures should be used.



The following are the appropriate Risk and Safety requirements:

Harmful if swallowed. Irritating to eyes, respiratory system, and skin. Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection. If swallowed seek medical advice immediately and show the product container.



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