Instructions for use

ROTI®DAB Kit

Substrate kit for detection of HRP activity on blot membranes and slides.

- High sensitivity
- DNAse-/RNAse-free
- For immunoblotting, immunohistochemistry and *in situ* hybridization

DAB substrate kit for detection of peroxidase (HRP) activity on immunoblots, immunohisto- and cyto-chemical slides and during *in situ* hybridization. ROTI®DAB Kit contains substrate, buffer solution and activation reagent, therefore including all three solutions necessary for HRP detection reaction. Application is very simple - all three kit solutions are diluted equally in water, and the blotting filter membrane, or the slides, are then incubated in this substrate solution. Since the solutions are delivered in drop dispensers, dosing is most convenient.
DAB is oxidized by HRP, forming a brown precipitate, which is insoluble in aqueous and organic solvents. This precipitate can be detected in visible light and does not bleach during long-term storage.
Addition of the DAB Metal Enhancer (Art. No. 9204) doubles the signal strength and shifts the colour to a lilac-gray, highly contrasted staining. Slides may be permanently mounted in hydrophilic, or, after dehydration, in hydrophobic mounting medium.

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The kit contains:
Red cap: DAB solution (Art. No. 9869),
10 ml Diaminobenzidine solution.
Yellow cap: DAB buffer (Art. No. 9870),
10 ml Imidazole buffer solution
Purple cap: Activation reagent (Art. No. 9871),
10 ml Hydrogen peroxide solution.
Contents of this Kit may not be bought separately.

One kit is sufficient for staining of approx. 44 minigel blot membranes or 500 slides.

Application for tissue staining
Note: Prepare the staining solution within 10 mins. before use.

1. Pipet 2 ml of distilled water (or DAB Metal Enhancer) into a reaction tube.
2. Add one drop each of DAB solution, DAB buffer, and Activation reagent and mix well.
3. Incubate tissue sections with the prepared staining solution at room temperature until suitable staining develops.
   Development times should be determined by the investigator, but generally an incubation of 2-10 mins. provides good staining intensity.
4. Wash the sections in distilled water in order to stop the detection reaction.

Application for blotting (Mini gels)
Note: Prepare the staining solution within 10 mins. before use.

1. Put 10 ml of distilled water (or DAB Metal Enhancer) into a reaction tube.
2. Add 225 µl each of DAB solution, DAB buffer, and Activation reagent and mix well.
3. Incubate the blotted and assayed transfer membrane in the prepared staining solution under agitation at room temperature for 10-60 mins. until suitable staining of the expected bands develops.
4. Wash the membrane under running water in order to stop the detection reaction.
5. Air dry membrane.

Please Note:
- Unclean equipment may cause false positive results by reacting with the staining solution. Therefore, we recommend to wash lab wares with water, then soak them in 1 M sulphuric acid for at least 30 mins. Wash thoroughly with deionized water and dry.
- The colour of the staining solution will change with time as result of oxidation. Prepare the staining solution immediately before use, and start the reaction within 10 minutes.
- Due to the high sensitivity of the ROTI®DAB staining, some background may appear. We recommend optimizing assay conditions beforehand, such as incubation times, blocking reagent, concentration of primary and secondary antibody and so on.
- In case the DAB Metal Enhancer is used, an even stronger background may appear. We recommend minimizing the time span used for the staining reaction.
- Stainless steel trays may cause precipitation of the dye. We recommend use of plastic trays instead.
- It is important to wash the membrane after the staining. Remaining staining solution may increase the background significantly.
- Keep the assayed membrane in the dark. Bands may fade if exposed to strong light.

Further Recommended Products:
DAB Metal Enhancer (Art. No. 9204)

Storage:
Store at +4 °C. Shipped on cool packs

Danger H302-H341-H351-H360D-H373

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