

# Instructions for use

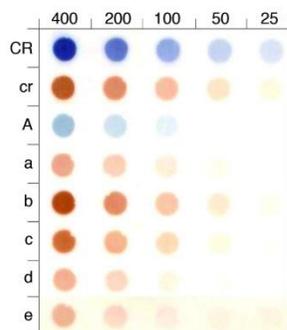


## DAB Metal Enhancer

**Ready-made solution for enhancement of HRP signals on blot membranes and slides.  
RNase-free, DNase-free**

DAB metal enhancer is used to enhance the signal strength in peroxidase (HRP) detections and to modify the signal colour from brown to purplish gray. Applications include all immunochemical assays like immunohistochemistry and -cytochemistry as well as Western blotting, but DAB metal enhancer may also well be used during *in situ* hybridization. When the DAB metal enhancer is applied, the sensitivity of DAB staining is about two times higher than with DAB alone. The purplish gray precipitate resulting from the staining reaction can be detected in visible light and does not bleach during long-term storage. Since it is insoluble in aqueous and organic solvents, slides may be permanently mounted in hydrophilic, or, after dehydration, in hydrophobic mounting medium.

One bottle for 100 ml is sufficient for enhancement of approx. 10 minigel blots or 100 slides.



**Figure:** Dot blot of a HRP conjugated antibody (400, 200, 100, 50 and 25 pg).

CR/cr: Detection using ROTI®DAB Kit, A-e: Detection using kits and DAB substrates from other suppliers. CR: Detection under supplementation with DAB Metal Enhancer, A: Supplementation with NiCl<sub>2</sub>.

### Application for tissue staining

**Note:** Prepare the staining solution within 10 mins. before use.

1. Pipet 2 ml of DAB Metal Enhancer into a reaction tube.
2. Add DAB reagents\* 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.

\*For instance one drop each of DAB Solution, DAB Buffer, and Activation Reagent of ROTI®DAB Kit, or freshly activated DAB solution.

### Application for blotting (Mini gels)

**Note:** Prepare the staining solution within 10 mins. before use.

1. Put 10 ml of DAB Metal Enhancer into a reaction tube.
2. Add DAB reagents\* and mix well.
3. Incubate the blotted and assayed transfer membrane in the prepared staining solution under agitation at room temperature for 5-60 mins. until suitable staining of the expected bands develops.
4. Wash the membrane under running water for at least 10 mins. in order to stop the detection reaction.
5. Air dry membrane.

\*For instance 225 µl each of DAB Solution, DAB Buffer, and Activation Reagent of ROTI®DAB Kit, or freshly activated DAB solution.

### Please Note:

- Unclean equipment may cause false positive results by reacting with the staining solution. Therefore, we recommend to wash lab ware 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.

- Signal enhancement by DAB Metal Enhancer may cause an even stronger background. We recommend minimizing the time span used for 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:

ROTI®DAB Kit (Art. No. 9202)  
DAB, powdered (Art. No. CN75)  
ROTI®Stock 10x PBS (Art. No. 1058)  
ROTI®Stock 10x TBS (Art. No. 1060)  
Hydrogen peroxide 30 % (Art. No. 9681)

**Storage:** Store at approx. +20 °C.

⚠ **Danger** H317-H334-H341-H350-H360D-H372-H412  
P273-P280-P302+P352-P308+P313

Full text of hazard- and precautionary statements  
see material safety data sheet section 2.2

## DAB Metal Enhancer

9204.1

100 ml

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