

Cat. # MK210

For Research Use

TaKaRa

TaKaRa DAB Substrate

Product Manual

v201402

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I. Description

The TaKaRa DAB Substrate includes reagents needed to prepare DAB (3,3'-diaminobenzidine tetrahydrochloride salt) solution, a substrate of horseradish peroxidase (HRP). DAB develops a dark brown-colored product upon reaction with HRP, and therefore can be used to visualize where peroxidase-conjugated antibodies bind to a sample. DAB is used in immunohistochemical staining and can also be used to stain other materials, including fixed cells and nitrocellulose membranes.

II. Components (500 assays)

Reagent A	: DAB color reagent	2.4 ml x 1
Reagent B	: Substrate buffer	30 ml x 2
Tube	: Tube with graduations	1
Tip (for Reagent B)	: Colored tip for Reagent B bottle	1
Dropper bottle		1

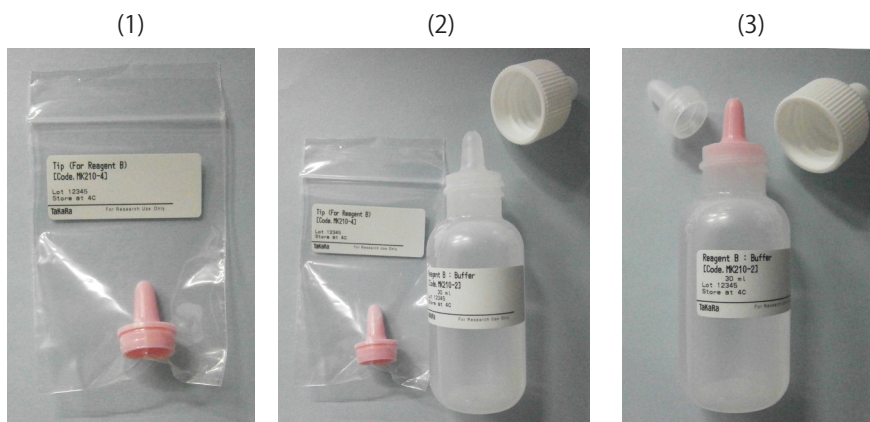


[Tube]

The tube is used for mixing Reagent A and Reagent B to prepare DAB substrate solution.

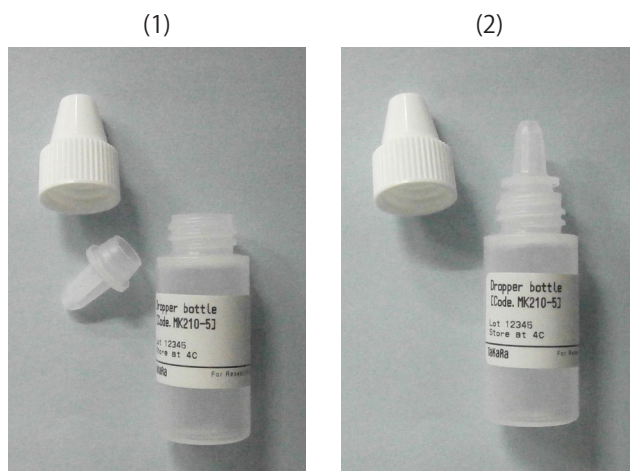
[Tip]

The Reagent B bottle comes with a semitransparent tip. This tip is not suitable for preparing small amounts of DAB substrate, as this tip cannot dispense drops. When preparing a small amount of DAB substrate solution, replace the tip with the included Tip (for Reagent B). The Tip (for Reagent B) has a narrower opening and can release the solution in drops.



[Dropper bottle]

The Dropper bottle can be used to store and dispense DAB substrate. The tip can be removed by pushing from the side, and DAB substrate can be transferred to the bottle.



※ Re-use

The Tube, Tip, and Dropper bottle can all be reused after washing with purified water and drying completely.

III. Storage 4°C

IV. Preparation of DAB Substrate

Add one drop of Reagent A (about 20 μ l) for each 500 μ l of Reagent B. Use the graduations on the Tube to measure the volume of the Reagent B. Cap the Tube and mix well without creating bubbles.*1

The prepared DAB substrate solution can be used directly, or the solution can be transferred from the Tube to the Dropper bottle before use.

After preparation, store at 2-8°C protected from light. The solution can be stored for two weeks.*2

- *1 : If bubbles are present after mixing, remove the bubbles before using the solution.
- *2 : Do not use DAB substrate solutions that were prepared on different days in the same experiment.

V. Protocol

- This product is used to detect HRP-conjugated secondary antibodies in immunohistochemical staining.
 - Bring the DAB substrate solution to room temperature before use.
 - To properly evaluate staining results, perform appropriate negative controls (e.g., use PBS instead of the primary antibody).
1. Dispense the DAB substrate solution onto the tissue section on a slide. Use approximately 4 drops ($\sim 100 \mu\text{l}$) per sample. Ensure that the tissue section is covered completely with the solution and incubate for 5 - 20 minutes at room temperature.
 2. Adjust the reaction time by comparing color development with the negative control by microscopy.
 3. After sufficient color development, dip and wash the slide in water.
 4. After dehydration using ethanol and xylene, cover with a non-aqueous mounting medium and coverslip.

VI. Precautions for Use

- Prevent the reagents from coming into contact with eyes, skin, and clothing. Be careful not to accidentally ingest or inhale the reagents.
- Handle Reagent A with great caution as it includes DAB, a potential carcinogen.
- Handle Reagent B with care as it contains weak hydrogen peroxide content.
- Dispose of DAB substrate appropriately.

VII. Related Products

TaKaRa POD Conjugate For Mouse Tissue/For Tissue (Cat. #MK200 - MK205)

NOTE: This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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