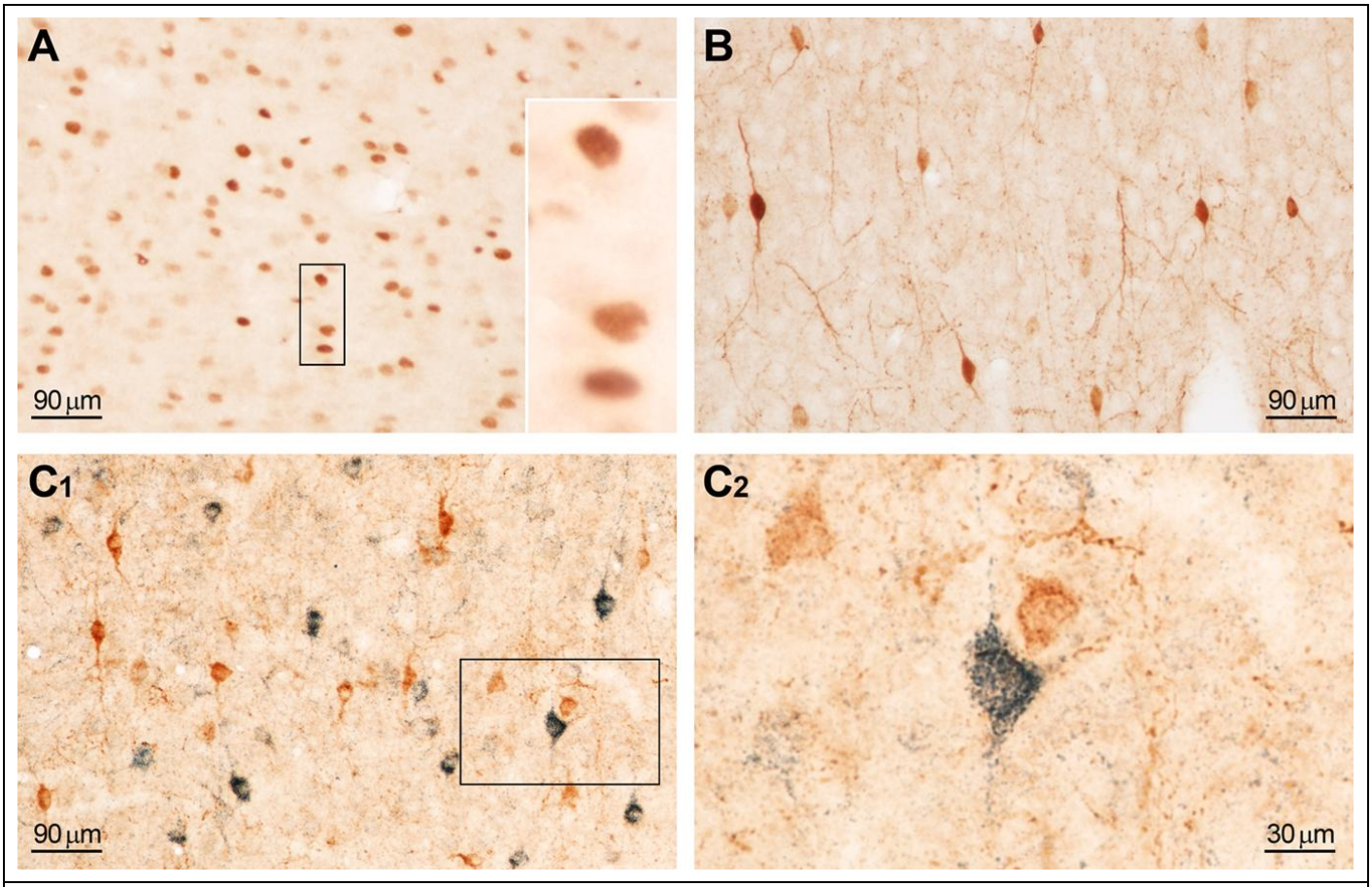
3, 3'-diaminobenzidine (DAB) Peroxidase Substrate Kit

(Laboratory Use Only, Store at 2-8 ºC)

3,3'-diaminobenzidine (DAB) is a chromogen popularly used in immunohistochemistry, immunocytochemistry, and immunoblotting. The Bioenno DAB Substrate Kit is ideal for immuno-labeling on tissue sections/cells to detect the activity of peroxidase (HRP). The brown DAB-HRP reaction product is alcohol and heat resistant, and is also suitable for electron microscopy (EM). The kit contains all of the necessary reagents to prepare about 330 ml of substrate working solution, and the stock solutions are contained in convenient dropper bottles. The kit can be stored in a dark area at 2-8ºC and is stable for 12 months.



Bioenno DAB Substrate Kit employed together with Bioenno BDHC Substrate Kit

(A,B) The DAB Substrate Kit was used in single-labeling immunohistochemistry. (C) Dual-labeling immunohistochemistry employing both DAB (brown) and BDHC (blue) kits. DAB was used as the first chromogen and BDHC was the second one. The boxed areas were magnified (63×) to highlight immuno-labeled neurons. Images were taken from adult rat brains.

References:

- Hsu SM, Soban E. Color modification of diaminobenzidine (DAB) precipitation by metallic ions and its application for double immunohistochemistry. J Histochem Cytochem 1982; 30:1079–1082.
- Trojanowski JQ, Obrocka MA, Lee VM. A comparison of eight different chromogen protocols for the demonstration of immunoreactive neurofilaments or glial filaments in rat cerebellum using the peroxidase-antiperoxidase method and monoclonal antibodies. J Histochem Cytochem 1983; 31:1217–1223.
- Chu NM, Janckila AJ, Wallace JH, Yam LT. Assessment of a method for immunochemical detection of antigen on nitrocellulose membranes. J Histochem Cytochem 1989; 37:257–263.

Warranty: 12 months from the date of purchase.
Return Policy: Bioenno Tech's return policy for this product is 90 days from the date of purchase.
Free Technical Support: Email your questions to ***contact@bioenno.com***

REAGENTS PROVIDED WITH THE KIT:

- **Buffer**: 12 ml of Stock Buffer in dropper bottle.
- **DAB**: 10 ml of DAB Stock Solution in dropper bottle.
- **H_2O_2**: 10 ml of Hydrogen Peroxide (H_2O_2) Solution in dropper bottle.

INSTRUCTIONS FOR USE (FOR TISSUES OR CELLS):

1. Finish the incubation with a peroxidase (HRP) detection system (*e.g.*, perform standard avidin-biotin-peroxidase immunohistochemistry), and then wash the tissues/cells in 0.01M PBS-T (0.01M PBS containing 0.3% Triton X-100) (pH 7.4 ± 0.1) for 15 min with 2-3 changes of the PBS-T.

2. Prepare DAB substrate working solution immediately before use (5 ml as an example):
   a. To 5 ml of distilled water (dH_2O), add 5 drops (approximately 200 µl) of **Buffer** and mix well;
   b. Add 3-5 drops (approximately 80-130 µl) of **DAB** stock solution and mix well;
   c. Add 3-5 drops (approximately 120-200 µl) of **H_2O_2** solution and mix well.

   The amount/drops of DAB and H_2O_2 can be adjusted and should be optimized by the investigator. Drop volumes differ due to solvent compositions.

3. Incubate the tissues/cells in freshly prepared DAB substrate working solution at room temperature (18-25ºC) for 8-12 min. Stop the reaction by transferring tissues/cells to dH_2O for seconds. Optimal reaction times should be determined by the investigator.

4. Wash the tissues/cells in 0.01M PBS-T (pH 7.4 ± 0.1) for 15 min (change the PBS-T 2-3 times during the washing), and then mount in the same PBS-T. Dehydrate and clean as usual. Coverslip with a non-aqueous mounting medium such as the Permount® mounting medium.

NOTES:

We recommend using glass-distilled water in the preparation of the substrate buffer. Deionized water may contain inhibitors of the peroxidase reaction. Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used to dilute the peroxidase substrate.

Variations in color intensity of the stock and working solutions may be seen between lots of this product. These variations will not affect the product stability or the intensity of the staining.

Prepare the substrate working solution immediately before use.

Slides developed with DAB can be dehydrated, cleared, and permanently mounted.

STORAGE, SAFETY, AND HANDLING PRECAUTIONS:

Store the kit in a refrigerator (2-8ºC). Avoid storing reagents or working solution in strong direct light.

DAB is a suspected carcinogen. Wear gloves, appropriate eye and face protection, and suitable protective clothing while using this reagent. Neutralize the solution/waste with potassium permanganate-sulfuric acid solution or chlorine bleach, and collect for hazardous waste disposal.

Avoid inhalation and contact with skin and eyes while handling. In case of contact, wash immediately and thoroughly with water and seek medical advice if necessary.