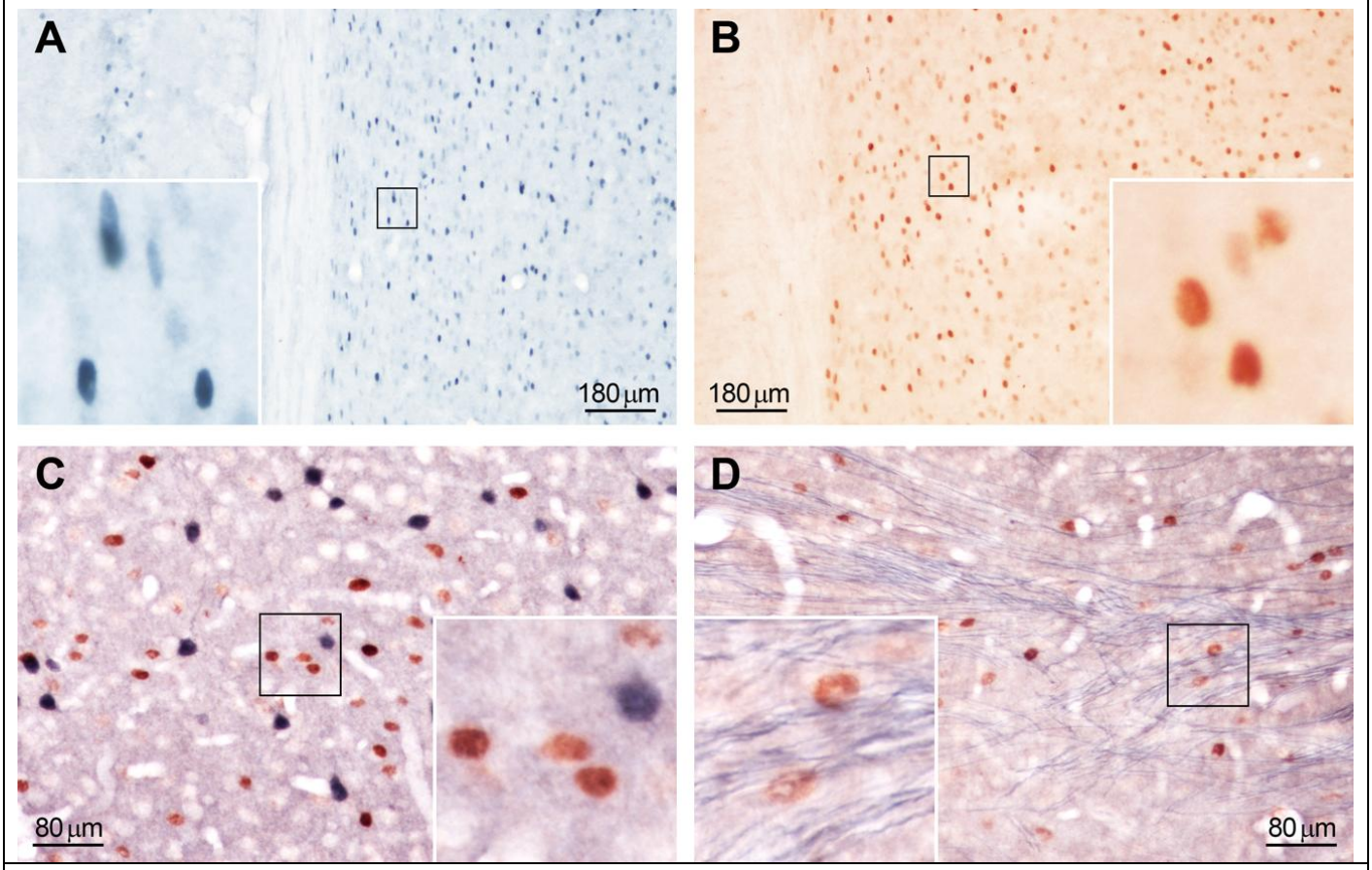
Cobalt-enhanced DAB Peroxidase Substrate Kit

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

The Bioenno DAB-Cobalt Substrate Kit is an advanced version of the DAB (3,3'-diaminobenzidine) Substrate Kit. This kit is ideal for single and/or double labeling immunohistochemistry/immunocytochemistry and can be used on either tissue sections or cells. The incorporation of cobalt into DAB can modify the color of the normally brown DAB reaction, leading to a distinct dark blue/bluish black color. The dark blue DAB-Cobalt reaction product is stable and can be easily distinguished from the brown DAB reaction product (see images and references below). 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.



Immunohistochemistry using the Bioenno DAB-Cobalt Substrate Kit

(A) The substrate working solution contains both DAB and Cobalt. The reaction product is dark blue. (B) Cobalt was not added into the substrate working solution. (C,D) Double-labeling immunohistochemistry: A brown reaction product was developed with the substrate solution containing DAB, and dark blue product was developed with the solution containing both DAB and Cobalt. These immunoreaction products can be distinguished on the same brain sections. DAB was used as the first chromogen and DAB-Cobalt was the second one. The boxed areas were magnified (63×) to highlight the DAB and DAB-Cobalt labeled neurons.

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 (pH 7.4 ± 0.1) in dropper bottle.
- **DAB**: 10 ml of DAB Stock Solution in dropper bottle.
- **Cobalt**: 10 ml of Cobalt 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-Cobalt 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. Continually add 3-5 drops (approximately 120-200 µl) of **Cobalt** stock solution and mix well;
   d. Add 3-5 drops (approximately 120-200 µl) of **H_2O_2** solution and mix well.

   *If a regular brown DAB reaction product is desired, simply ignore the addition of **Cobalt** stock solution. The amount/drops of DAB, Cobalt, 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-Cobalt substrate working solution at room temperature (18-25ºC) for 8-12 min. Stop the reaction by transferring tissues/cells to dH_2O for 2-5 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 a total 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 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 in diluting 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-Cobalt or 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 and Cobalt are suspected carcinogens. Wear gloves, appropriate eye and face protection, and suitable protective clothing while using these reagents. 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.