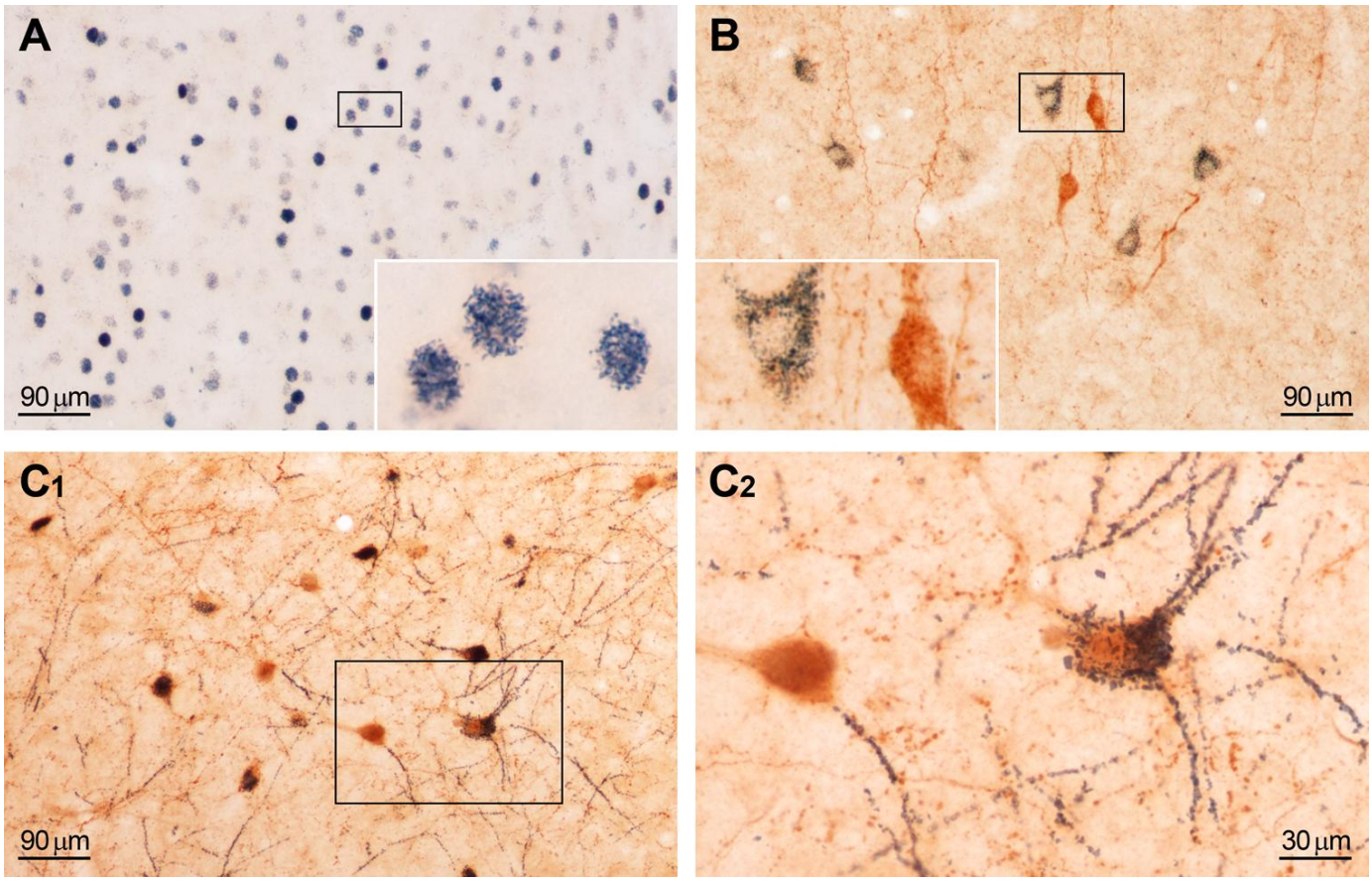
Benzidine Dihydrochloride (BDHC) Peroxidase Substrate Kit

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

BDHC is a very sensitive chromogen and can produce a **blue** and **particulate** reaction product in the presence of peroxidase (HRP) enzyme under acidic conditions. The Bioenno BDHC Substrate Kit contains the reagents necessary to prepare an acidic buffer (pH 6.2 ± 0.1) (1,800 ml) and a BDHC substrate working solution (280 ml). The kit is ideal for **dual-labeling** immunocytochemistry/immunohistochemistry, and can be used together with 3,3'-diaminobenzidine (DAB), the most popularly used chromogen. The blue/particulate reaction product of BDHC is easily distinguished from the brown/diffuse reaction product of DAB (see images and references below). The kit has been tested on tissue sections and cells derived from mice and rats. The BDHC substrate is also suitable for electron microscopy (EM).



Bioenno BDHC/DAB Substrate Kits are used for single- and double-labeling immunohistochemistry

(A) BDHC Substrate Kit was employed and the boxed area was magnified (100×) to highlight the BDHC-labeled neurons. (B,C) Double-labeling using chromogens BDHC (blue) and DAB (brown) to present single- and/or dual-labeled neurons. DAB was used as the first chromogen and BDHC was the second one. The boxed areas were magnified (63×) to highlight the immuno-labeled neurons. The images were taken from adult rat brains.

References:

- Lakos S, Basbaum AI. Benzidine dihydrochloride as a chromogen for single- and double-label light and electron microscopic immunocytochemical studies. J Histochem Cytochem 1986; 34:1047–1056.
- Levey AI, Bolam JP, Rye DB, Hallanger AE, Demuth RM, Mesulam MM, Wainer BH. A light and electron microscopic procedure for sequential double antigen localization using diaminobenzidine and benzidine dihydrochloride. J Histochem Cytochem 1986; 34:1449–1457.
- Mendez I, Elisevich K, Flumerfelt B. Dopaminergic innervation of substance P-containing striatal neurons by fetal nigral grafts: an ultrastructural double-labeling immunocytochemical study. J Comp Neurol 1991; 308:66–78.

- Mikkelsen JD, Larsen PJ, Sørensen GG, Woldbye D, Bolwig TG, Hastings MH, Ebling FJ. A dual-immunocytochemical method to localize c-fos protein in specific neurons based on their content of neuropeptides and connectivity. Histochemistry 1994; 101:245–251.
- Chen Y, Fenoglio KA, Dubé CM, Grigoriadis DE, Baram TZ. Cellular and molecular mechanisms of hippocampal activation by acute stress are age-dependent. Mol Psychiatry 2006; 11:992–1002.

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:

- **15× Acidic Buffer**: 60 ml × 2 Qty, 15× stock solution (pH 6.2 ± 0.1).

  The 15× stock buffer should be diluted with distilled water (dH_2O) to obtain 1× working solution. For example, first warm up the 15× stock solution to room temperature (18-25ºC), and then add 60 ml of the stock to 840 ml of dH_2O.

  The **1× acidic buffer**/working solution will be used prior to the reaction of BDHC as well as for washing and mounting of tissues/cells after the BDHC reaction.

- **Reaction Diluent**: 10 ml of Reaction Diluent **Stock Solution** in dropper bottle.
- **BDHC**: 10 ml of BDHC **Stock Solution** in dropper bottle.
- **H_2O_2**: 10 ml of Hydrogen Peroxide 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 well as usual (*e.g.*, wash in 0.01M PBS containing 0.3% Triton X-100, pH 7.4 ± 0.1 for 15 min), followed by a wash in **1× acidic buffer** (pH 6.2 ± 0.1) for a total of 15-20 min. Change the acidic solution 1-2 times during the washing.

2. Prepare BDHC 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 **Reaction Diluent** and mix well;
   b. Add 3-5 drops (approximately 120-200 µl) of **BDHC** Stock Solution and mix well;
   c. Add 3-5 drops (approximately 120-200 µl) of **H_2O_2** Solution and mix well.

   The optimal amount/drops of BDHC and H_2O_2 should be determined by the investigator.

3. Incubate the tissues/cells in freshly prepared BDHC substrate working solution on ice or at room temperature (18-25ºC) for 3-6 min. Stop the reaction by transferring tissues/cells to 1× acidic buffer. Optimal reaction times should be determined by the investigator.

4. Wash the tissues/cells in 1× acidic buffer for 10-15 min (change the washing buffer 2-3 times during the washing), and then mount in the same 1× acidic buffer. Dehydrate and clean as usual. Coverslip with a non-aqueous mounting medium such as the Permount® mounting medium.

NOTES:

PBS or PBS-T that is simply adjusted by acids cannot replace the kit-provided acidic buffer.

Avoid long-term wash/storage in 1× acidic buffer (pH 6.2 ± 0.1), which may result in the washout of BDHC reaction products. The blue and granular precipitates can be reapplied if washed out (re-develop in the BDHC substrate solution to achieve the most desirable results).

BDHC reaction products can be completely removed in buffers with a pH value larger than 7.0. Please use BDHC as the second chromogen in double immuno-labeling.

STORAGE, SAFETY, AND HANDLING PRECAUTIONS:

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

BDHC solution contains benzidine dihydrochloride, which is a suspected carcinogen. Wear gloves, appropriate eye and face protection, and suitable protective clothing while using this reagent. Neutralize the solution with potassium permanganate-sulfuric acid solution or chlorine bleach, and collect any waste from this solution 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.