Bioenno Tech, LLC

BDHC Substrate Kit

Catalog Number: 003850

Benzidine Dihydrochloride (BDHC) Peroxidase Substrate Kit

(Laboratory Use Only, Store at 2-8 °C)

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 (see references below). 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 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).

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![Image A](image1.png) ![Image B](image2.png)

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

(A) Standard avidin-biotin-peroxidase immunohistochemistry was performed, and BDHC Substrate Kit was employed. 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.

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**References:**


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:

- **15x Acidic Buffer**: 60 ml × 2 Qty, 15x stock solution (pH 6.2 ± 0.1).
  The 15x stock buffer should be diluted with distilled water (dH2O) to obtain 1x working solution. For example, first warm up the 15x stock solution to room temperature (18-25°C), and then add 60 ml of the stock to 840 ml of dH2O. The 1x 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.

- **H2O2**: 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 1x acidic buffer (pH 6.2 ± 0.1) for a total of 10-20 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 (dH2O), 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 H2O2 Solution and mix well.

   The optimal amount/drops of BDHC and H2O2 should be determined by the investigator.

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

4. Wash the tissues/cells in 1x acidic buffer for 10-15 min (change the washing buffer 2-3 times during the washing), and then mount in the same 1x 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 1x 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 (2-8°C). 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.