EZLabel™ Antibody HRP Labeling Kit

(Store at -20°C)

Cat. No. K4310-1, contains sufficient reagents to label 1 mg of antibody

i. **Introduction:**

Horseradish Peroxidase is widely used as an enzymatic label in immunochemistry assays such as ELISA. Preparing stable and reproducible antibody-HRP conjugates is one of the biggest challenges of developing immunoassays. The BioVision's EZLabel™ Antibody HRP Labeling Kit utilizes a novel chemistry to generate highly reproducible IgG-HRP conjugates with a simple procedure. The resulting conjugates have been shown to be extremely stable, retaining 94% activity after storage for 95 days at 37º when stored at a concentration of 0.5 µg/ml. The HRP-labeled antibody can be directly used for multiple downstream applications including ELISA, Immunohistochemistry, Immunoassays, etc.

ii. **Applications**:

- HRP labeled antibodies can be used for ELISA, western blot, Immunohistochemistry, Immunoassays, etc.

iii. Kit Contents:

Components	K4310-1	Part Number
EZLabel™ IgG Activator (25x)	10 µl	K4310-1-1
EZLabel™ HRP (20 mg/mL)	60 µl (1.2 mg)	K4310-1-2
EZLabel™ Quenching Reagent (1x)	25 µl	K4310-1-3

iv. User Supplied Reagents and Equipment:

- 1X Phosphate Buffered Saline (1X PBS), pH 7.2-7.5, dH_2O, Desalting columns (Zeba Spin Desalting Columns from ThermoFisher)

v. **Reagent Preparation and Storage Conditions:**

Store the kit at -20°C. Read the entire protocol before performing the experiment. Briefly spin small vials prior to opening. Bring the kit components to room temperature before use.

- **EZLabel™ IgG Activator (25x) -** Store at -20ºC. Dilute EZLabel™ IgG Activator (25x) with dH_2O to recommended dilution. Keep the vial in the desiccated container. Dilute EZLabel™ IgG Activator (25x) with dH_2O to the recommended dilution. Prepare 1x or 0.1 x diluted IgG Activator from 25x IgG Activator in deionized water:
  a) Measure at least 1 mg of the 25x IgG Activator by weight on an accurate analytical balance, using an appropriate pipettor with a disposable tip to deliver the liquid into a tared Eppendorf or comparable tube.
  b) For 1x dilution add 25 µLof dH_2O to each mg of Activator weighed out.
  c) For 0.1x dilution add 250 µLof dH_2O to each mg of Activator weighed out.
  d) Immediately vortex to mix the activator thoroughly.

vi. Antibody HRP Labeling Protocol:

- **Antibody Solution Preparation:** The IgG to be labeled should be at a concentration 1.0 -10.0 mg/ml in pure 1X PBS and should not contain any preservatives or carriers such as sodium azide, Proclin 300 or BSA.
- **HRP:IgG Molar Ratio:** The recommended HRP:IgG molar ratio for most conjugations reaction is 4:1. However, lower or higher ratios may give better results depending upon the antibody characteristics and the intended end-use. Conjugates for ELISA may perform optimally at a different HRP:IgG molar ratio than conjugates to be used for immunohistochemistry. Please refer table below.
- **Labeling Reaction:**
  1. Desalt IgG into 1X PBS, pH 7.2 – 7.4. Measure the absorbance of the IgG solution at 280 nm using PBS as a blank. Divide the A280 by 1.40 to obtain the IgG concentration in mg/ml.
  2. Remove the EZLabel™ IgG Activator (25x) from the freezer. Allow sufficient time to allow the container and contents to come to room temperature before opening the outer vial. **Note:** The vial containing the IgG Activator can be removed from the freezer up to 24 hours before use.
     For less than 1 mg of IgG: Dilute EZLabel™ IgG Activator to 0.1x in deionized water. 40 uL of 0.1x EZLabel™ IgG Activator solution is required per mg of IgG.
     Measure the IgG Activator by weight. To prepare 0.1X IgG Activator, add 250 uL of dH_2O to each mg of IgG Activator weighed out.
     For more than 1 mg of IgG: Dilute EZLabel™ IgG Activator to 1x in deionized water: 2 uL of 1x EZLabel™ IgG Activator is required per mg of IgG.
     Measure the IgG Activator by weight. To prepare 1X Activator, add 25 uL of dH_2O to each mg of IgG Activator weighed out.
     Note: Diluted IgG Activator must be used within 5 minutes of preparation. If more than 5 minutes passes before use, discard the solution and prepare a fresh solution.
  3. Immediately vortex to mix the activator thoroughly and then add appropriate amount of EZLabel™ IgG Activator per mg of IgG.
  4. Incubate the solution at room temperature for 1 hour with gentle mixing or shaking.
     Note: End-over-end mixing is ideal, but other types of gentle mixers or shakers can be used. A longer incubation is not harmful and even overnight incubations will be successful.
  5. Desalt the IgG into pure 1x PBS. We recommend desalting spin columns with a 40 Kd MW cutoff for small volumes of IgG. Use of gravity desalting columns and extensive washing with centrifugal filter units is also acceptable.

6. Quantitate the concentration and amount of activated IgG. The IgG concentration should be greater than 0.8 mg/ml.
   Note: The activated IgG is stable and can be stored at 2-8ºC for at least 1 month.
7. Calculate the volume of EZLabel™ HRP required for your desired HRP:IgG ratio (see table below under HRP:IgG Molar Ratio). Add the calculated volume of EZLabel™ HRP to the IgG solution.

   Note: Mix gently at room temperature for 18-24 hours. End-over-end mixing is ideal, but other types of gentle mixers or shakers can be used.
8. Remove the EZLabel™ Quenching Reagent from the freezer. Allow it to reach room temperature before opening the vial.
9. Add 0.2 uL of Quenching Reagent per ul of EZLabel™ HRP added to the reaction.
10. Mix gently at room temperature for 1 hour. Reaction can be incubated for longer than 1 hour or overnight.
11. To improve performance, purify the conjugate from the unincorporated HRP and reaction components by size exclusion chromatography.

HRP:IgG Molar Ratio: The recommended HRP:IgG molar ratio for most conjugations reaction is 4:1. However, lower or higher ratios may give better results depending upon the antibody characteristics and the intended end-use. Conjugates for ELISA may perform optimally at a different HRP:IgG molar ratio than conjugates to be used for immunohistochemistry. The table below shows the conversion from molar ratio to mass ratio and the volume of activated HRP required per mg of IgG for each molar ratio.

HRP:IgG Molar Ratio	HRP:IgG Mass Ratio	Vol. Activated HRP per mg of IgG
1 : 1	0.3 : 1	15 µL
2 : 1	0.6 : 1	30 µL
3 : 1	0.9 : 1	45 µL
4 : 1 (recommended)	1.2 : 1	60 µL
5 : 1	1.5 : 1	75 µL
6 : 1	1.8 : 1	90 µL
8 : 1	2.4 : 1	120 µL

VII. RELATED PRODUCTS:

Biotin Quantitation Kit (Colorimetric) (K811)
Biotin-LC-LC-NHS (2346)
Biotin-PEG_4-amine (2791)
Annexin V-FITC Apoptosis Kit (K101)
Annexin V-FITC Reagent (1001)
Human IgG (1296)
Rabbit IgG (1268)
Annexin V-Biotin Apoptosis Kit (K109)
Annexin V- Cy5 Apoptosis Kit (K103)
Annexin V- PE Apoptosis Detection Kit (K128)
Red Fluorescent Protein R-PE (R-Phycoerythrin) (6005)
EZlabel™ Antibody FITC Labeling Kit (K831)
EZlabel™ Antibody Cy5 Labeling Kit (K838)
EZlabel™ Antibody Cy3 Labeling Kit (K836)
EZ-Desalt™ Spin Desalting Columns (6564)
Biotin-LC-NHS (2345)
Biotin-NHS (2347)
Biotinylated Bovine Serum Albumin (Biotin-LC-BSA) (7099)
Hi-Bind™ Protein A-Agarose (6520)
Hi-Bind™ Albumin-IgG Depletion Beads (7933)
Mouse IgG (1265)
Hi-Bind Ni QR Agarose Beads (6562)
Annexin V-Cy3 Apoptosis Kit (K102)
Annexin V-EGFP Apoptosis Kit (K104)
Annexin V-PE-Cy5 Apoptosis Detection Kit (K129)
Red Fluorescent Protein Monoclonal Antibody (3984)
EZlabel™ Protein FITC Labeling Kit (K832)
EZlabel™ Protein Cy5 Labeling Kit (K839)
EZlabel™ Protein Cy3 Labeling Kit (K837)
EZlabel™ Protein Biotin Labeling Kit (K835)

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