EZLabel™ Antibody RPE Labeling Kit

(Store at Multiple Temperatures)

Cat. No. K4313-1, contains sufficient reagents to label 1 mg of antibody

i. **Introduction:**

R-Phycoerythrin is widely used as a fluorescent label in immunochemistry assays such as ELISA and in more complex techniques such as flow cytometry. Preparing bright, stable and reproducible antibody-PE conjugates is one of the biggest challenges of developing bead-based immunoassays and high quality reagents for flow cytometry. The BioVision's RPE-IgG conjugation kit utilizes a novel chemistry to generate bright and highly reproducible RPE-IgG conjugates with a simple procedure. The resulting conjugates have been shown to be extremely stable, retaining 95% activity after storage for 30 days at 37º with concentrations as low as 0.5 µg/ml.

Applications:

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

ii. **Kit Contents:**

Components	K4311-1	Part Number	Storage Temperature
EZLabel™ IgG Activator (25x)	10 µl	K4313-1-1	-20ºC
EZLabel™ RPE (20 mg/mL)	80 µl (1.6 mg)	K4313-1-2	4ºC
EZLabel™ Quenching Reagent (1x)	25 µl	K4313-1-3	-20ºC

iii. **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)

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

- **EZLabel™ IgG Activator (25x):** Store at -20ºC. Keep the vial in the desiccated container. Dilute EZLabel™ IgG Activator (25x) with dH_2O to the recommended dilution. Prepare 1x 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) Add 24 µL of dH_2O to each mg of Activator weighed out.
  c) Immediately vortex to mix the activator thoroughly.
- **EZLabel™ RPE (20 mg/mL):** Store at 4ºC. **Do Not Freeze.**
- **EZLabel™ Quenching Reagent (1x):** Store at -20ºC.

v. **Antibody RPE 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.
- **RPE:IgG Molar Ratio:** The recommended RPE:IgG molar ratio for an initial conjugation reaction is 1:1. However, lower or higher ratios may give better results depending upon the antibody characteristics and the intended end-use. Conjugates for bead-based immunoassay platforms may perform optimally at a different RPE:IgG molar ratio than conjugates to be used for flow cytometry.. 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 1.4 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.
  3. Calculate volume of 1x IgG Activator required: 2 µl of 1x IgG Activator is required per mg of IgG. For small amounts of IgG, IgG Activator can be diluted an additional 10-fold (to 0.1x). In this case, 20 µl of 0.1 x IgG Activator solution is required per mg of IgG.
     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.
  4. Immediately vortex to mix the activator thoroughly and then add appropriate amount of EZLabel™ IgG Activator per mg of IgG.
  5. Incubate the solution at room temperature for 1 hour with gentle mixing or shaking.
  6. **Note:** End-over-end mixing is ideal, but other types of gentle mixers or shakers can be used. Reaction can be incubated for longer than 1 hour or overnight.
  7. 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.
  8. Quantitate the concentration and amount of activated IgG. The IgG concentration should be greater than 0.5 mg/ml.
     Note: The activated IgG is stable and can be stored at 2-8ºC for at least 1 month.
  9. Calculate the volume of EZLabel™ RPE required for your desired RPE:IgG ratio (see table below under RPE:IgG Molar Ratio). Add the calculated volume of EZLabel™ RPE 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.

10. Remove the EZLabel™ Quenching Reagent from the freezer. Allow it to reach room temperature before opening the vial.
11. Add 5 uL of Quenching Reagent per µl of EZLabel™ RPE added to the reaction.
12. Mix gently at room temperature for 1 hour. Reaction can be incubated for longer than 1 hour or overnight.
13. Test conjugate in the desired application. To improve performance, purify the conjugate by size exclusion chromatography.

RPE:IgG Molar Ratio:

RPE:IgG Molar Ratio	RPE:IgG Molar Ratio	RPE:IgG Molar Ratio
0.50 : 1	0.80 : 1	40 µl
0.75 : 1	1.20 : 1	60 µl
1.00 : 1*	1.6 : 1	80 µl
1.25 : 1	2.0 : 1	100 µl
1.50 : 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)

FOR RESEARCH USE ONLY! Not to be used on humans.