

R-PE Labeling Kit

Cat #: orb1098293 (manual)

Size: 10 µg (1 conjugation reaction)

For Research Use Only. Not For Use in Diagnostic Procedures!

- ✚ Store all kit components at 4°C upon arrival.
- ✚ Keep Activated R-PE in the dark, do not freeze.
- ✚ Components B and D may be frozen (-20°C) for best performance.
- ✚ The performance of the product is guaranteed for a minimum of 12 months when stored as directed.

Description

The R-PE Labeling Kit provides a convenient way to conjugate antibodies to R-Phycoerythrin (R-PE). R-PE belongs to the Phycobiliproteins family of highly soluble and fluorescent proteins derived from cyanobacteria and eukaryotic algae. R-PE is made of α , β and γ subunits and is present as $(\alpha\beta)_6\gamma$. The protein has broad absorption bands with peaks at 496 nm, 546 nm, and 565 nm, and can be excited with versatile excitation sources. The broad excitation spectrum provides the advantage for multi-color immunofluorescence staining and cell sorting. The R-PE Labeling Kit is a SH-reactive labeling kit. Modified R-Phycoerythrin can easily react with SH groups of a target antibody without the need for an additional activation, thus simplifying the conjugation protocol. Modified R-PE and sulfhydryl group on the antibody form a covalent bond during conjugation.

Figure 1.

Physical and Spectral Properties of Activated R-PE:

Molecular weight: 240 kDa

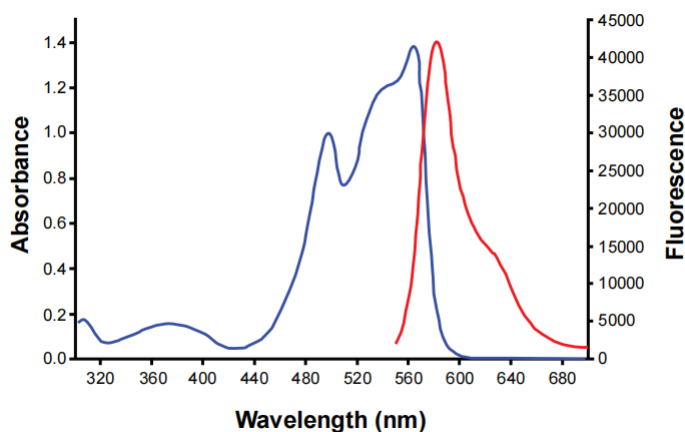
Absorption peaks: 499, 541, 565 nm

Maximum emission: 578 nm

— Absorption

— Emission

Storage: 4°C (Do *NOT* freeze!)



Required Materials

- PBS
- Microcentrifuge tubes

Features

- This kit is optimized for conjugating R-phycoerythrin (R-PE) to antibody.
- It provides sample materials to perform one conjugation reaction.

Kit Contents

Contents	orb1098293
Activated R-PE	5 μ l
Component B	10 μ l
DMSO	25 μ l
Component D	1 vial
Filtration Tube	1 tube

Quality Control

The quality of the R-PE Labeling Kit is tested on a lot-to-lot basis to ensure consistent product quality.

R-PE Labeling Kit Protocol

1. Preparing the antibody for conjugation

- 1.1 Add 100 μ l of 1X PBS (pH 7.2-7.4) and the sample solution containing 10 μ g of IgG to Filtration Tube.
- 1.2 Pipette to mix and centrifuge at 8,000 x g for 10 minutes.
- 1.3 Add an appropriate amount of 1X PBS (pH 7.2-7.4) to the upper surface of membrane to resuspend the IgG added to the reaction
- 1.4 Add 2 μ l of Component B per 10 μ l of IgG solution onto the membrane of Filtration Tube, and pipette to mix the IgG. Incubate the reaction at room temperature for 30 minutes.
- 1.5 Add 100 μ l of 1X PBS (pH 7.2-7.4), and centrifuge 8,000 x g for 10 minutes.
- 1.6 After discard the filtrate, add an appropriate amount of 1X PBS to the upper surface of membrane to resuspend the IgG added to the reaction.

2. Conjugation reaction

- 2.1 Add 2 μ l of activated R-PE per 10 μ g of reduced IgG onto the membrane of Filtration Tube, and pipette to mix. Incubate 1 hour at room temperature with agitation and protect from light.
- 2.2 Block excess free thiols
 - 2.2.1 Add 20 μ l of Component C into the vial of Component D.
 - 2.2.2 Add 0.6 μ l of mixture solution of conjugation mixture from Step 2.1 and mix completely. Incubate at room temperature for 30 minutes and keep away from light.
 - 2.2.3 Transfer the solution to a microcentrifuge tube (not included in this kit) and conjugation is complete. One to two Activated R-PE should be introduced into one reduced IgG molecule. Unconjugated Activated R-PE remained in the conjugation complex might cause background increase with immunoassay.

3. Storage of conjugates

- 3.1 Store the R-PE-labeled antibody at concentration > 0.5 mg/ml or add 1 mg/ml of BSA as a stabilizer.

3.2 Add preservative (e.g. 0.01% Sodium azide).

3.3 Store the R-PE-labeled antibody in the dark, at 4°C. Do NOT Freeze!

3.4 The R-PE-labeled antibody is shown to be stable for 6 months. However, the best storage conditions for any particular conjugate must be determined by experimentation, and depend on the starting antibody stability, and storage condition of kit components.

Related Ordering Information

Cat. No.	Description	Size
PC101-0500	1.5ml Microcentrifuge Tube, Clear, Conical, Lock Cap	500 pcs
CC711-0500	PBS, pH 7.4	500 ml

Caution

- ⚠ During operation, always wear a lab coat, disposable gloves, and protective equipment.
- ⚠ All products are for research use only.