INSTRUCTIONS

EZ-Link™ Plus Activated Peroxidase

31487 31488 31489

0506.4

Number	Description
31487	**EZ-Link Plus Activated Peroxidase,** 1mg
31488	**EZ-Link Plus Activated Peroxidase,** 5mg
31489	**EZ-Link Plus Activated Peroxidase Kit**

Kit Contents:

EZ-Link Plus Activated Peroxidase, 5 × 1mg

Sodium Cyanoborohydride Solution (5M), 1 × 0.5mL

Quenching Buffer, 1 × 25mL, contains 3M ethanolamine, pH 9

BupH™ Phosphate Buffered Saline Pack, 1 each, results in 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 when reconstituted with 500mL of ultrapure water

BupH Carbonate-Bicarbonate Buffer Pack, 1 each, results in 0.2M carbonate-bicarbonate, pH 9.4 when reconstituted with 500mL of ultrapure water

Note: EZ-Link Plus Activated Peroxidase contains 1-3 moles of aldehyde per mole of peroxidase.

Storage: Upon receipt store product protected from moisture at -20°C. Product is shipped with an ice pack.

Introduction

The Thermo Scientific™ EZ-Link™ Plus Activated Peroxidase is an amine-reactive horseradish peroxidase (HRP) that provides higher conjugate yields (> 95%) than glutaraldehyde chemistry. The protocol produces a stable conjugate in approximately 1-2 hours. Varying conjugation parameters, such as molar excess of peroxidase, buffer and pH affect HRP incorporation level. Polymeric conjugates with high enzymatic activity are formed at high pH, while lower molecular-weight conjugates are formed at near-neutral pH.

Important Product Information

- Do not use sodium azide as a preservative for buffers or the conjugate. Sodium azide is an inhibitor of HRP and could interfere with downstream applications.
- The included protocols use a ~4-fold molar excess of HRP to IgG. Adjust the molar-fold excess as needed for the specific downstream application.
- The kit contains two reaction buffers. Conjugation efficiency is greater when using the pH 9.4 Carbonate-Bicarbonate Buffer than when using the pH 7.2 Phosphate Buffered Saline (PBS).
- Purification of the HRP conjugate after coupling minimizes nonspecific binding in downstream applications. Remove non-conjugated HRP using the Thermo Scientific™ Pierce™ Conjugate Purification Kit (Product No. 44920). Alternatively, non-conjugated IgG can be removed using an appropriate chromatography column.

Procedure for Conjugating Activated Peroxidase to an Antibody at pH 9.4

1. Add 500mL of ultrapure water to the dry-blend Thermo Scientific™ BupH™ Carbonate-Bicarbonate Buffer.
2. Prepare 1mg of IgG in 0.5-1.0mL of Carbonate-Bicarbonate Buffer.

   Note: Free amino groups in the IgG preparation, such as Tris or glycine, will interfere with conjugation. If necessary, dialyze or desalt the IgG into the Carbonate-Bicarbonate Buffer to remove free amino groups.
3. Reconstitute 1mg of lyophilized EZ-Link Plus Activated Peroxidase with 100µL of ultrapure water and add it to the IgG solution or add the protein sample directly to the lyophilized activated peroxidase.

   Note: EZ-Link Plus Activated Peroxidase is more hydrophobic than non-modified HRP.
4. Incubate reaction for 1 hour at room temperature.

   Note: Desalting reaction mix into pH 7.2 conjugation buffer before the addition of Sodium Cyanoborohydride reductant may increase reduction of Schiff Base, stabilizing conjugation of HRP to protein.
5. In a fume hood, add 10µL of Sodium Cyanoborohydride and react at room temperature for 15 minutes.
6. Add 20µL of Quenching Buffer and react at room temperature for 15 minutes.
7. If desired, desalt, dialyze or purify the conjugate.
8. Store conjugate at 4°C for up to 4 weeks. For long-term storage, add one of the following: bovine serum albumin at 10mg/mL, an equal volume of glycerol or Pierce Peroxidase Conjugate Stabilizer (Product No. 31503). Prepare single-use aliquots and store at -20°C.

Procedure for Conjugating Activated Peroxidase to an Antibody at pH 7.2

1. Add 500mL of ultrapure water to the dry-blend Phosphate Buffered Saline (PBS).
2. Prepare 1mg of IgG in 0.5-1.0mL of PBS.

   Note: Free amino groups in the IgG preparation, such as Tris or glycine, will interfere with conjugation. If necessary, dialyze or desalt the IgG into PBS to remove free amino groups.
3. Reconstitute 1mg of lyophilized EZ-Link Plus Activated Peroxidase with 100µL of ultrapure water and add it to the IgG solution or add the protein sample directly to the lyophilized activated peroxidase.

   Note: EZ-Link Plus Activated Peroxidase is more hydrophobic than non-modified HRP.
4. In a fume hood, immediately add 10µL of Sodium Cyanoborohydride to the reaction and incubate for 1 hour at room temperature.
5. Add 20µL of Quenching Buffer and react at room temperature for 15 minutes.
6. If desired desalt, dialyze or purify the conjugate.
7. Store conjugate at 4°C for up to 4 weeks. For long-term storage, add one of the following: bovine serum albumin at 10mg/mL, an equal volume of glycerol or Pierce™ Peroxidase Conjugate Stabilizer (Product No. 31503). Prepare single-use aliquots and store at -20°C.

Related Thermo Scientific Products

44892	**AminoLink™ Reductant (sodium cyanoborohydride),** 2 × 1g
28372	**BupH™ Phosphate Buffered Saline Pack,** 40 packs
28382	**BupH Carbonate-Bicarbonate Buffer Pack,** 40 packs

General References

Porstmann, B., *et al.* (1985). Which of the commonly used marker enzymes gives the best results in colorimetric and fluorimetric enzyme immunoassays: horseradish peroxidase, alkaline phosphatase, β-galactosidase? *J Immun Meth* **79:**27-37.

Imagawa, M., *et al.* (1982). Characteristics and evaluation of antibody-horseradish peroxidase conjugates prepared by using a maleimide compound, glutaraldehyde, and periodate. *J Appl Biochem* **4:**41-57.

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