

Horseradish Peroxidase (HRP) Conjugation Kit

Product Description

Cat. Number	Name
U27381	Horseradish Peroxidase (HRP) Protein Labeling Kit incl. 2mg HRP; labels 1-2mg protein
U27382	Horseradish Peroxidase (HRP) Protein Labeling Kit incl. 3x200µg HRP; labels ca 3x200µg protein
Storage:	at 4°C (L) . This kit is stable for up to 1 year.

Introduction

HRP is widely used in ELISA/EIA as a detection molecule. HRP, a glycoprotein (40 kDa), is available in highly purified form, relative inexpensive, very stable, and provides several options (free amino, carboxyl, and carbohydrate groups) for covalent coupling to other antigens and antibodies. It has a very high turn over number and remains active after chemical modifications.

Conjugation of proteins (peptides, antigen or antibodies) requires activation of HRP and removal of activating agents. The activated HRP is then reacted with the protein. Interchim provide a unique method of HRP activation and keeping the activated HRP in lyophilized/liquid state for extended period of time. The preactivated HRP (supply in liquid or powder) is then directly mixed with other protein that have free amino group for an easy coupling. This kit therefore takes the time consuming and problematic steps out to provided a consistent and easy labeling of peptides and proteins. The labeling of proteins occurs via the activated amino groups of HRP to the amino groups of the coupling proteins.

[Directions for use](#)
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Directions for use

Handling and Storage, Kit contents

Precautions:

This kit contains preactivated HRP (powder or liquid) and it is stable for at least 6-months when kept at 4°C. Do not store HRP in solution or diluted forms. Preactivated HRP should be used immediately (within 30 min) upon reconstitution.

Reagents Provided in the Kit:

Preactivated HRP (1 vial of 2 mg powder/kit /##U27381, 3 vials of 200µg each of Lyophilized/kit #U27382)

Conjugation Buffer, 0.1 M NaHCO₃, pH 9.5 (10 X), 100 ml; (dilute 1:10 with water before use)

Blocking Buffer; 500 ul

Conjugate Stabilizer; 2 ml.

Dialysis bag (Cut off size 10-12 kda)

Note: If proteins of 10KDa or less are being coupled then it is a good idea to use membranes with lower cut-off such as (3 KDa). Wash the membrane thoroughly with 70% ethanol and then with distilled water.

Reagents Required But not Provided:

1. PBS, pH 7.4 for Dialyzing the conjugate – See [related products](#) cat#UP68723A.
(dissolve 0.26 g KH₂PO₄, 2.17 g Na₂HPO₄.7H₂O, and 8.71 g of NaCl in 1L H₂O).

Contact your local distributor Uptima, powered by

uptima@interchim.com  213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

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2. Clamps #CB-1050 for closing the dialyses membrane

Protocol ⁽¹⁾: HRP Conjugation Procedure

1. Dialyze the protein to be coupled in 1X conjugation buffer (0.1 M NaHCO₃, pH 9.5) extensively.

Note: The protein concentration should be 1-4 mg/ml. If the protein is in pure form or in saline or in H₂O, it is possible to adjust the pH by adding 10X conjugation buffer and omitting dialysis. If the protein is dry powder in water or PBS then it is possible to reconstitute it in directly in conjugation buffer.

2. Add 0.5-1 ml of dialyzed or pre-equilibrated protein (up to 1-2 mg) and add it to the vial containing 2mg preactivated HRP. Mix it at room temp. for 3 Hr or overnight at 4°C.

Note: Typically, 1 mg of antigen or antibody can be coupled to 2 mg HRP (1:2 Ag/Ab:HRP) as a starting point. It is possible to vary this ratio to 1:1 or 2:1 etc and test the resulting conjugate. This ratio will determine the working dilution of the conjugate, background, and also the sensitivity in a given technique. It is difficult to predict what ratio of the antigen/antibody and HRP will work the best.

3. Add Blocking Buffer (200 µl/ml of conjugate). Mix it for 1 Hr at RT.

4. Dialyze the conjugate extensively against PBS at 4°C.

Note: It is possible to test the conjugate without dialyses as the conjugate is typically used at 1:1K-1:50K depending upon the antibody titer.

5. Add stabilizing buffer to the dialyzed conjugate (add 1 ml for each 500 ul of starting protein volume).

6. The conjugate can be kept at 4°C for up to 6 months.

Storage of the Conjugate

The stabilizer already has a bacteriostatic agent. Do not add sodium azide (inhibits peroxidase). The conjugate should be kept at 4°C for 6 months or it can be stored frozen in suitable aliquots. Avoid freezing and thawing. Glycerol (50%) can be added and conjugate kept at -20°C without freeing.

Recommended Usage of the Conjugate

Antigens that are coupled to HRP can be used as detection probes in inhibition or competitive assays.

Antibody conjugated to HRP are used typically as detection probes in sandwich type immuno-assays.

ELISA testing of the Conjugate:

Interchim supplies both the single component TMB substrate for testing the conjugate ([#UP66478](#)) and all of the components for ELISA (see [related products](#)).

If an antigen is coupled to HRP- the antigen-HRP conjugate can be tested using appropriate antibody coated plates (coat 1-5 µg/ml) and blocked. Incubate the conjugate (1:1K-100K) for 1-Hr at room temp, wash, and then add TMB-substrate for 15-min. Blue color is obtained in positive well. Stop reaction with 1N H₂SO₄ (blue color turns yellow).

Read the yellow color at 450 nm.

If antibody is coupled to HRP- test the conjugate as above except that the antigen-coated plates should be used.

High background in ELISA is usually due to the use of **excessive** conjugate, improper conjugate diluents or blocking of coated antigens.

The HRP-conjugate dilution will depend upon the strength of the antigen or antibody. Weaker the antibody titer is, lower the conjugate dilution will be. Typically, if HRP conjugate is used at 1:1K or less then background could become a problem. The HRP-conjugate can be purified to remove the unbound antigen or antibody to improve the working of the conjugate.

Free HRP in conjugate is usually not a problem as it will not bind to the antigen or antibody and removed during washing.

Technical and Scientific Information

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uptima@interchim.com  213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

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Related / associated products and documents

- >peroxidase substrates TMB solutions for ELISA #[UP66478](#), Check #[UPS0817](#) and Aqueous #[UPS0818](#), other [Uptima reagents for ELISA](#)
 TMB solution for Blotting and IHC #[295885](#)
 Stop solution for TMB, #[UPS29590](#)
 opMetal Enhanced DAB kit #[679921](#) [for blotting and IHC]
 DAB ready to use solution #[UP73232](#)
- >Desalting [CelluSep dialysis tubings](#) (CelluSep H1), [Dialysis Clamps](#)
 Desalting columns #[UP84874](#)
- Buffers PBS buffer (Phosphate Buffer Saline) #[UP68723A](#)
 ELISA Plate Coating buffer concentrate (10X) #[J0719A](#)
 Antibody and Conjugate Diluents for ELISA (10X) #[I94790](#)
 Wash buffer concentrate (20X) for ELISA #[I98050](#) .
 Stop solution for TMB substrate (ELISA)#[S29590](#)
- Plates High binding ELISA plates #[Q89414](#) (8 wellsx12 strips), #[Q89403](#) (96 wells)
- #[BG7670](#) protein (antigen/antibodies) Biotinylation Kit, 1 kit
- [PH-BZ0000](#) Products Highlights
 see [BioSciences Innovations catalogue](#) and [e-search tool](#).

Ordering information

Catalog size quantities and prices may be found at <http://www.interchim.com>.
 Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

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