Protocol

Conjugation of Antibodies to Horseradish Peroxidase

Eric A. Berg and Jordan B. Fishman

Antibodies conjugated with horseradish peroxidase (HRP) are one of the most widely used bioreagents in the biological sciences. This protocol is a basic method for adding HRP to a thiolated antibody and can be adapted for use with different cross-linkers. Conjugation methods usually focus on linking through the lysines on HRP because there are only six of them and their modification does not adversely affect enzyme activity.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

Antibody of interest
Dimethyl sulfoxide (DMSO)
Horseradish peroxidase
Hydroxylamine buffer <R>
N-Succinimidyl *S*-acetylthioacetate (SATA)
Phosphate-buffered saline (PBS)
Sephadex G-25
Succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (SMCC)

Equipment

Chromatography equipment
Dialysis tubing (10,000 MW cutoff)
Rotator
Stir plate

From the Antibodies collection, edited by Edward A. Greenfield.

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Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot099309

METHOD

Addition of SATA to Antibody

Ideally, one can use the same antibody with protected SATA and begin at Step 4.

1. Dissolve SATA to 10 mg/mL in DMSO immediately before use.
2. Add 1 μ L of SATA per milligram of antibody. Use 1–10 mg/mL antibody in buffer (pH 6.5–7.5).
3. Mix the contents and incubate the reaction for 30 min at room temperature. The activated antibody can be stored at 4°C until needed.
4. To deprotect the thiol groups, add 1 volume of hydroxylamine buffer to every 10 volumes of the antibody solution. Mix the contents and incubate the reaction for 2 h at room temperature.
5. Desalt the solution using a G-25 sizing column. Proceed immediately to the next step to minimize disulfide formation.

Preparation of Maleimide:HRP

6. Weigh out 1.1 mg of HRP for each milligram of thiolated antibody. Add 1.1 mg of SMCC per milligram of HRP. Add PBS to a final concentration of 8 mg/mL HRP. Mix for 2 h on a rotator.
7. Desalt the HRP on a G-25 column.

Preparation of Ab:HRP Conjugate

8. Immediately mix the HRP with the thiolated antibody at 1 mg of HRP per milligram of antibody. Incubate with mixing for 16–20 h at 4°C.
9. Dialyze against three changes of PBS for 2 h at room temperature.
It may be necessary to separate free enzyme from conjugate using ion exchange or affinity chromatography.
10. Sterilize the enzyme–antibody conjugate solution by filtration. Store for up to 30 d at 4°C. For longer storage, lyophilize and store for up to 1 yr at –70°C. Reconstitute immediately before using in the desired application.

See Troubleshooting.

TROUBLESHOOTING

Problem (Step 10): There is low HRP activity when using the labeled antibody.

Solution: This means that there is sodium azide in the solution. Remove it before application.

Problem (Step 10): There is low antibody binding when using the labeled antibody.

Solutions:

- Use less SATA.
- Use an alternative cross-linker (e.g., EDC).

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RECIPE

Hydroxylamine Buffer

Reagent	Quantity
Hydroxylamine	0.5 M
EDTA	25 mM
PBS (pH 7.2–7.5)

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