Attach a protein onto a gold surface

TR0002.2

Introduction

Researchers often need to immobilize proteins on inert surfaces for varied applications including isolation and purification of target macromolecules, selective removal of contaminants, and enzymatic catalysis and chemical modification. Immobilized enzymes on gold foil electrodes have been used widely in affinity biosensors for the capture and detection of macromolecules for analytical purposes.

Chemical cross-linking is perhaps the most commonly used method for immobilizing proteins on inert surfaces. Following is a protocol for attaching a protein onto a gold surface using the chemical cross-linker dithiobis(succinimidyl propionate) (DSP, Product No. 22585). DSP is a homobifunctional, amine-reactive cross-linker. The linkage formed between DSP and the gold surface is very stable, exceeding the strength and stability of covalent silane bonds with glass. The disulfide linkage in DSP chemisorbs rapidly to gold surfaces, while the active NHS groups on either end of DSP are reactive toward primary amine groups in proteins.

Materials Required

- DSP (Product No. 22585), 4 mg required per ml of DMSO coating solution
- Dimethylsulfoxide (DMSO, Product No. 20684), sufficient volume to cover and later rinse gold surface
- Protein solution: 1-2 mg/ml protein dissolved in a compatible buffer (pH 7-8, containing at least 50 mM NaCl). Recommended buffers include PBS (Product No. 28372), MOPS and HEPES. Avoid buffers containing primary amines, such as Tris and glycine.

  Note: This procedure can be used to couple other ligands besides proteins that contain primary amines. For molecules that are not soluble in aqueous buffers, coupling can be performed in acetone, isopropyl alcohol or other solvents like DMSO, as long as amines are maintained in an uncharged state by addition of triethylamine or triethanolamine at a concentration equal to or greater than the concentration of DSP.

Procedure

1. Weigh DSP into a tube, and dissolve it in DMSO. Use 4 mg DSP per ml of DMSO necessary to cover the gold surface.
2. Incubate gold foil in dissolved DSP for 30 minutes at room temperature.
3. Rinse gold foil with DMSO and then with water. Gold foil is now activated with NHS groups.

   Note: Proceed directly to the next step; if protein coupling is not performed immediately, the NHS reactive groups will hydrolyze and protein coupling will be negligible.
4. Immediately add the protein solution to the activated gold foil and incubate for 1-4 hours at room temperature.

   Note: Incubation for longer time will not adversely affect conjugation efficiency. However, conjugation does not advance significantly after the first 1-2 hours.
5. Rinse the surface with buffer to remove cross-linker by-products (NHS leaving groups) and unconjugated protein.

   Note: Free NHS groups (by-products of conjugation and hydrolysis) absorb strongly at 280 nm. Therefore, take care if attempting to determine the conjugation efficiency by measuring the change in protein concentration in solution.

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Related Thermo Scientific Pierce Products

21578 **DTSSP**, 50 mg, water-soluble form of DSP

20036 **Bioconjugate Techniques**, 2nd edition, Greg T. Hermanson, Academic Press, Inc., 2008.

Additional Information

Crosslinker selection guides and detailed instructions for individual products are available from our web site. A previous version of this Tech Tip suggested using isopropyl alcohol to dissolve and coat the DSP on the gold surface, as directed in the original publication by Katz[1] and cited in the book by Hermanson et al.[2] In practice, however, DSP is not soluble in isopropyl alcohol; therefore, DMSO has been suggested as a more suitable solvent, in keeping with subsequent work by Katz. We offer several of the crosslinker used by Katz in his experiments with gold electrodes. Water-soluble linkers or disulfide chemicals (e.g., cystamine) other than DSP may be used to functionalize gold surfaces without using DMSO as the solvent.

References

1. Katz, E.Y. (1990). *J. Electroanal. Chem*. **291**, 257
2. Hermanson, G. T., Mallia, A. K., and Smith, P. K. (1992). Immobilized Affinity Ligand Techniques, Academic Press Inc., p. 59

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.