TECH TIP # 1

Attach a protein onto glass, silica, or quartz surface using a cleavable crosslinker

TR0001.2

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Introduction

Researchers frequently develop assay and affinity purification methods for specialized applications and instrumentation. Commonly, these methods require immobilizing specific proteins, protein-containing cell surfaces and phages or other ligands to a surface material. Consequently, our technical support scientists are frequently contacted by customers seeking methods for immobilizing proteins or other ligands to glass surfaces. A related Tech Tip (#5) describes protocols for attaching an antibody to glass through reduced sulfhydryls. This Tech Tip presents two additional protocols for immobilizing such molecules by a crosslink that can be reversed. The covalently immobilized molecule may be cleaved from the surface by reduction of the disulfide-containing crosslink. The method requires two Thermo Scientific Pierce® Products: an aminosilane reagent for derivatizing a glass surface to present primary amines, and a versatile crosslinker capable of effecting cleavable amine-to-amine or amine-to-sulfhydryl linkages between the derivatized glass and a molecule of interest.

Note: throughout the protocols, "protein" refers to the molecule being attached, although any molecule with the appropriate amine or sulfhydryl groups can be substituted.

Materials Required

- Aminosilane Reagent: 3-Aminopropyltriethoxysilane (Product No. 80370)
- Acetone (solvent/diluent for Aminosilane Reagent)
- Coupling Buffer: PBS-EDTA (50 mM Phosphate, 0.15 M NaCl, 10 mM EDTA, pH 7.2). Use BupH™ Phosphate Buffered Saline Packs (Product No. 28372) and add EDTA to a concentration of 10 mM. Alternatively, other neutral or slightly alkaline amine-free buffers such as borate, HEPES, and bicarbonate also may be used but add at least 10 mM EDTA to prevent metal-catalyzed oxidation of sulfhydryls during the final crosslinking step. Avoid sulfhydryl-containing components in the buffer, as these will react with the pyridyl disulfide portion of the crosslinker.
- Crosslinker: Sulfo-LC-SPDP (Product No. 21650)
- Reducing Agent: Dithiothreitol, DTT (Product No. 20290)

Procedure for Amino-Silylating the Surface Material

1. Thoroughly wash and dry the glass, silica or quartz surface to be coated.

   Note: Perform Steps 2 and 3 in a fume hood.

2. Prepare 2% solution of Aminosilane Reagent in acetone. For example, mix 1 part Aminosilane Reagent with 49 parts anhydrous acetone. Prepare a volume sufficient to immerse or cover the surface material.
3. Immerse surface material in the diluted reagent for 30 seconds.
4. Rinse surface with additional acetone.
5. Allow surface to air-dry.

   Note: The dried silylated surface may be stored for later use. For amine-to-surface attachment of the protein, continue with Protocol 1. For sulfhydryl-to-surface attachment of the protein, continue with Protocol 2.

Protocol 1: Amine-to-Surface Attachment of the Protein

A. Separately modify silylated surface and protein with the crosslinker Sulfo-LC-SPDP

1. Prepare Coupling Buffer as indicated in Materials Required section.
2. Dissolve protein at a concentration of 10 mg/ml in Coupling Buffer.
3. Prepare a 10 mM solution of Sulfo-LC-SPDP by dissolving 5.2 mg/ml in water.

   Note: This solution is needed in steps 4 and 5, which must be performed immediately and simultaneously.

4. Add 50 µl of the Sulfo-LC-SPDP solution to 1 ml of protein solution. Mix and set aside to react for at least 30 minutes at room temperature.
5. Add 50 µl of the Sulfo-LC-SPDP solution per 1 ml of Coupling Buffer and mix. Cover the silylated surface with this solution and allow the reaction to proceed for at least 30 minutes at room temperature.

   Note: longer incubation times with either the protein or silylated surface will not adversely affect the efficiency of modification.

B. Purify the modified protein and reduce the modified surface

1. Purify the modified protein from reaction byproducts by dialysis or gel filtration into Coupling Buffer.
2. Rinse the modified surface with Coupling Buffer to remove reaction by-products.

   Note: If desired, the surface can be dried and stored desiccated at 4°C for later use.

3. Reduce the modified surface by covering it for 30 minutes with a solution consisting of 8 mg DTT dissolved per 1 ml of Coupling Buffer (= 50 mM DTT solution).
4. Remove the DTT solution from the surface and save. If desired, the concentration of the pyridine-2-thione released can be determined by measuring the absorbance at 343 nm of the removed solution, although for surface modifications, the concentration of this leaving group may be too small to measure effectively. (For details, see the instructions that accompany Sulfo-LC-SPDP, Product No. 21650).
5. Rinse the reduced surface thoroughly with Coupling Buffer to remove any residual DTT.

   Note: If desired, the surface can be dried and stored desiccated at 4°C for later use.

C. Crosslink the protein to the surface

1. Cover the reduced surface with the modified protein solution. Allow reaction to proceed for 18 hours (e.g., overnight) at room temperature or 4°C.
2. Remove protein solution.

   Note: This removed solution is the non-conjugated protein fraction; depending on the protein amount used, it may be possible to perform a protein assay on this fraction compared to a pre-coupled fraction to determine the efficiency of conjugation.

3. Rinse surface with Coupling Buffer to remove residual non-conjugated protein.

Protocol 2: Sulfhydryl-to-Surface Attachment of the Protein

A. Modify the silylated surface with Sulfo-LC-SPDP

1. Prepare Coupling Buffer as indicated in Materials Required section.
2. Prepare a 10 mM solution of Sulfo-LC-SPDP by dissolving 5.2 mg/ml in water.
3. Immediately add 50 µl of the Sulfo-LC-SPDP solution per 1 ml of Coupling Buffer, mix, and then cover the silylated surface with this solution. Allow the reaction to proceed for at least 30 minutes at room temperature.

   Note: longer incubation time will not adversely affect the efficiency of modification.
4. Rinse the SPDP-modified surface with Coupling Buffer to remove reaction byproducts and non-reacted crosslinker.

   Note: If desired, the surface can be dried and stored desiccated at 4°C for later use.

B. Ensure availability of free sulfhydryls on protein

1. Assess the availability of free sulfhydryls on the protein using Ellman's reagent (Product No. 22582) according to the product instructions.
2. If necessary, make sulfhydryls available on the protein by reducing the protein for 30 minutes in 50 mM DTT; then purify the reduced protein from the reductant by gel filtration (see Related Pierce Products). Alternatively, use Immobilized TCEP Disulfide Reducing Gel (Product No. 77712) according to the product instructions.

   Note: Be aware that proteins may be inactivated by reduction of disulfide bonds.

C. Crosslink protein to the surface

1. Cover the SPDP-modified surface with sulfhydryl-containing protein solution. Allow the reaction to proceed for 18 hours (e.g., overnight) at room temperature or 4°C.
2. Remove the protein solution, which contains non-conjugated protein. Depending on the ratio of protein to surface used, it may be possible to perform a protein assay on this fraction compared to a pre-coupled fraction to determine the conjugation efficiency.
3. Rinse surface with Coupling Buffer to remove residual non-conjugated protein.

Procedure for Cleaving the Crosslinked Protein After its Use on the Surface

1. Incubate the surface-bound proteins with 10-50 mM DTT at pH 8.5 for 15-30 minutes at 37°C. If necessary, the stringency of the disulfide spacer arm reduction can be optimized to minimize any adverse reduction of the protein or protein complex being reclaimed.
2. Alternatively, if the protein is to be examined by denaturing SDS-PAGE, the disulfides can be cleaved by boiling the protein-containing surface for 5 minutes in SDS-PAGE sample loading buffer (2% SDS, 0.25 mM Tris base, 10% glycerol) containing 5% 2-mercaptoethanol (Product No. 35601) or 100 mM DTT.

Related Thermo Scientific Pierce Products

22582	**Ellman's Reagent**, 5 mg
89891	**Zeba™ Desalt Spin Columns**, 5 × 5 ml columns for processing 500-2,000 µl samples
77712	**Immobilized TCEP Disulfide Reducing Gel**, 5 ml
35601	**2-Mercaptoethanol**, 6 × 1 ml
20036	**Bioconjugate Techniques**, 2nd Edition, Greg T. Hermanson, Academic Press, Inc., 2008.

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.