Application Note B11.0

Enzyme and Protein Immobilization with SiliaBond Maleimide

The use of enzyme has shown great promises in molecular biology and in a wide range of applications from analytical biochemistry to innovative biomolecular-mediated synthesis and catalysis. An increasing number of chemical functionalities can be used to rapidly immobilize enzymes without sacrificing their activity.

Depending on the enzyme functionalities accessible for grafting, SiliCycle offers different **SiliaBond** functionalized silica particles that can selectively react with active biomolecules. For exemple, the **SiliaBond Maleimide** has shown a great efficiency for the immobilization of biomolecules bearing free sulfhydryl (-SH) groups.

One of the persisting problems of sulfhydryl-bearing proteins is their ability to form dimmers which prevent optimal immobilization. A common way to avoid such issue is the use of reducing agents (dithiothreitol, 2-mercaptoethanol or 2-mercaptoethylamine) that allow complete breakage of the disulfide bond responsible for the protein dimerization prior to reaction with the **SiliaBond Maleimide** reactive surface.

STANDARD PROTOCOL FOR IMMOBILIZATION OF PROTEIN ON SILIABOND MALEIMIDE

General Considerations

The SiliaBond Maleimide can be use in any polar solvent (water, methanol, DMF) which does not contain any free sulfhydryl moieties that would otherwise inhibit and reduce conjugation efficiency. The extend exposure to highly basic (pH > 10) or acidic (pH < 2) will lead to the accelerate degradation of the silica particles and is highly not recommended.

Introduction of Sulfhydryl Groups Through Amine Modification

Biomolecules that do not contain any sulfhydryl group but have amine moiety can be modified using N-Succinimidyl Sacetylthioacetate (SATA) or 2-Iminothiolane. HCl (Traut's reagent) and be successfully immobilized with the **SiliaBond Maleimide**.

Ligand Solution Preparation

A 10-20 mg/mL ligand (biomolecule) solution is prepared by the sequential solubilisation and homogenization of the ligand in PBS buffer (0.1 M sodium phosphate (Na_2HPO_4) and 0.15 M NaCl at pH 7.2).

Reduction of Disulfide Bond Prior to Immobilization

1) Reducing the buffer: Combine 100 µL of 1 M sodium phosphate buffer pH 6.0 (Na_2HPO_4), 5 µL of 0.5 M EDTA and 900 µL of Ultrapure Water (18 MΩ).

2) Mix 2 mL of the protein solution with 6 mg of 2-mercaptoethylamine and incubate for 2 hours at 37°C.

3) Cool the solution to room temperature and remove the 2-mercaptoethylamine using either a desalting column or a centrifugal filter with the proper molecular weight cut-off.

Immobilization

1) Suspend 250 mg of **Silia*Bond* Maleimide** in 10 mL of PBS (0.1 M sodium phosphate (Na_2HPO_4) and 0.15 M NaCl at pH 7.2).

2) Combine with 10 mL of the ligand solution and gently mix.

3) Stir the reaction for at least 2 hours at 4°C to prevent degradation of the ligand.

4) Wash the complexed **Silia*Bond* Maleimide** with PBS, 1 M NaCl, water and store at 4°C in an aqueous buffer containing a preservative (0.02% sodium azide).

Notes:

This protocol is provided as a typical protocol for the immobilization of biomolecular ligands. Further improvement in conjugation efficiency can be achieved for other biomolecular ligands through tuning of the reaction conditions (pH, temperature, buffer, concentration, ligand and supported reagent concentrations).

SILICYCLE RECOMMENDED PRODUCT:

R71030B **Silia*Bond* Maleimide**, 40-63 µm, 60 Å (also available in SPE and 96-well plates upon request)