Corning® Sulfhydryl-BIND™ Surface Plates

Biomolecular Immobilization Protocol

CORNING

Introduction

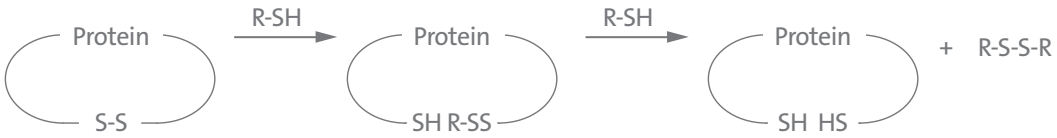
Corning's Sulfhydryl Binding Plates and Strips have a Maleimide surface that is intended to immobilize biomolecules through available -SH moieties, usually from a cysteine residue. Reduction of disulfide bonds may be necessary if sulfhydryl groups are not readily accessible. Alternately, a primary amine may be modified with Traut's reagent to add a sulfhydryl group. The maleimide reaction forms a covalent linkage with a biomolecule that is site-specific, which is beneficial when specific orientation of the immobilized biomolecule is necessary for the retention or optimization of activity—immunological, enzymatic, etc. This surface has proved very useful for the following applications: 1) the site-directed immobilization of reduced antibodies and antibody fragments [$F(ab')_2$], 2) immobilization of peptides and proteins with available cysteine residues, and 3) the immobilization of biomolecules, such as proteins and DNA, that can be modified to possess an available sulfhydryl group.

Sample preparation for the Maleimide surface may involve either the reduction of disulfide bonds or the modification of primary amine groups with 2-iminothiolane HCl (Traut's reagent) in order to introduce sulfhydryl groups to biomolecules lacking this functional group.

Reduction of Disulfide Bonds

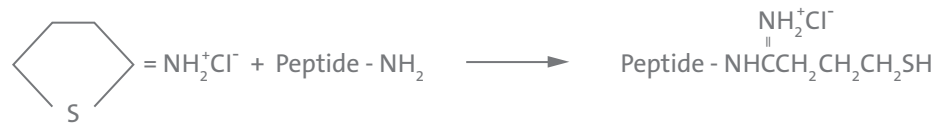
Disulfide bonds are formed between two cysteine residues on a protein. These units are relatively unreactive. Activation may be achieved by the reduction of disulfide bonds to sulfhydryl groups. Common reducing agents include 1) dithiothreitol (DTT), 2) 2-mercaptoethanol, 3) 2- mercaptoethylamine, and 4) tris (2-carboxyethyl)-phosphine, hydrochloride (TCEP-HCl). The mild reducing agents, DTT for example, will reduce only the exposed bonds and will not affect those buried within the molecular structure, thereby preserving the integrity of the biomolecule.

Reducing Agent = R-SH



Conversion of Amine Groups to Sulfhydryl Groups

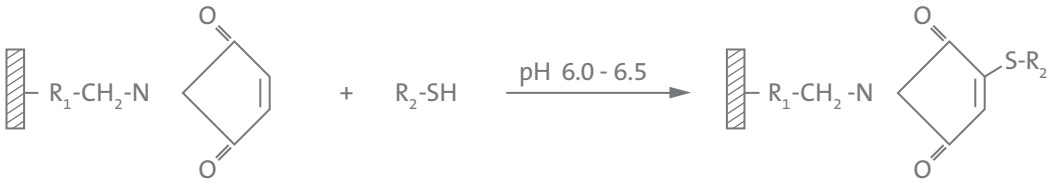
There are several methods of introducing free sulfhydryls onto biomolecules. One method involves converting an amine to a sulfhydryl group using 2-iminothiolane or Traut's reagent[4].



Traut's Reagent

The free sulfhydryl groups formed by either the reduction of disulfide bonds or by the modification of amine groups on existing amino acid side chains which can revert and form unwanted disulfide bonds by oxidation. The addition of EDTA will help solve this problem by chelating any oxidizing metals in the solution.

Free sulfhydryl groups are covalently immobilized to the maleimide surface in the following manner:



Maleimide Surface Biomolecule Immobilization

R_1=Spacer arm R_2=Protein, DNA, etc.

Covalent Coupling Procedure

1) Dilute biomolecule possessing an available sulfhydryl group (SAMPLE) to 1-100 µg/mL in an appropriate buffer pH 6.5 containing 1 mM ethylenediaminetetraacetic acid (EDTA). (Phosphate buffered saline pH 6.5 has been shown to be a suitable buffer; buffer must be free of any sulfhydryl containing compounds). If the SAMPLE does not possess an available sulfhydryl group, but disulfide bonds are available, the following buffers may be used:

   a) PBS pH 6.5 supplemented with 1mM EDTA and 0.0001 mM dithiothreitol (OTT) or,

   b) PBS pH 5.0 supplemented with 1mM EDTA and 0.1 m M Tris(2 -carb oxyethyl)-phosphine, hydrochloride (TCEP-HCI).

   NOTE 1: The maleimide reaction proceeds best and specifically with -SH groups at a slightly acidic pH of 6.0 to 6.5.

   NOTE 2: The EDTA is added to the reaction buffer to chelate any oxidizing metals present in the buffer that may cause the formation of unwanted disulfide bonds.

   NOTE 3: DTT and TCEP-HCI are mild reducing agents that are used to reduce disulfide bonds to form free sulfhydryl groups that can react with the maleimide surface.

   NOTE 4: Traut's reagent (2-iminothiolane) is a compound that reacts at pH 7-10 with epsilon or N-terminal amine groups by introducing a charged spacer arm with an available -SH group on the exposed end. Dilute the protein or biomolecule to be modified to desired concentration (1-10 µg/mL) in 15 mM Traut's reagent + 50 mm Tris, 150 mM NaCl, 1 mM EDTA, pH 7.6. Incubate for 1 hour at room temperature. Add the modified SAMPLE to the maleimide surface without additional purification or dilution.

2) Add 100 µL of SAMPLE to each well. Be sure to leave a well blank as a control.

3) Incubate for 1 hour at room temperature.

4) Decant the solution and rinse 3 times with an appropriate wash buffer without detergent. (PBS has been shown to be an adequate wash buffer).

5) Block the remaining active sites with a conventional blocker which must be a protein blocker containing available sulfhydryl groups. A detergent blocker is not sufficient. (0.2% nonfat dry milk has been shown to be an adequate blocker for this surface). An incubation time of 30 minutes is recommended.

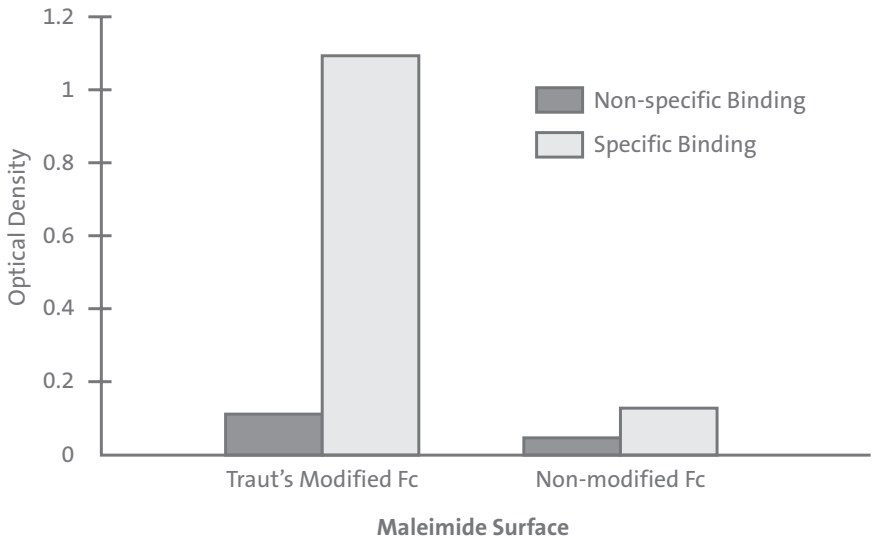
6) Decant the solution. Do not rinse.

7) Dilute subsequent reagents in an appropriate buffer containing 10% normal serum. (PBS pH 7.2 to 7.4 with 10% fetal bovine serum has been shown to be a suitable diluent). The addition of 10% normal serum to this diluent has been shown to reduce non-specific background binding.

8) Proceed with the remainder of the assay or procedure being performed. No other special requirements exist.

Performance Criteria

The Maleimide surface is tested for site-specific immobilization of sulfhydryl groups in the following manner. Using a Goat IgG Fc fragment that is either modified or non-modified with Traut's reagent, specific immobilization through sulfhydryl groups is detected with a Horse Radish Peroxidase labeled Rabbit anti-Goat IgG Fc antibody. This site-specific immobilization of Traut's modified Fc is demonstrated in the following graph.



The results indicate that there is a 7-fold increase in optical density at 405 nm for Traut's modified Fc as compared to non-modified Fc immobilized on the Maleimide surface, which is a clear indication that the immobilization of Fc to this surface is site-specific for sulfhydryl groups.

References

1. Butler, John E. Immunochemistry of Solid-Phase Immunoassay. Boca Raton, Florida: CRC Press, Inc. (1991).
2. Engvall E and Perlmann P. Immunochemistry. 8:871 (1971).
3. Maggio ET. Enzyme-Immunoassay. Boca Raton, Florida: CRC Press, Inc. (1980).
4. Pierce 1993 Immunotechnology Catalog & Handbook.
5. Tijssen P. Practice and Theory of Enzyme Immunoassays in Laboratory Techniques in Biochemistry and Molecular Biology. Vol. 15. Ed. R.H. Burdon and P.H. van Knippenberg, New York: Elsevier Biomedical (1985).
6. Wong Shan S. Chemistry of Protein Conjugation and Crosslinking. Boca Raton, Florida: CRC Press, Inc. (1991).

For additional product or technical information, visit **www.corning.com/lifesciences** or call 800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

CORNING

Corning Incorporated
Life Sciences
836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476
www.corning.com/lifesciences

ASIA/PACIFIC

Australia/New Zealand
t 61 427286832

Chinese Mainland
t 86 21 3338 4338
f 86 21 3338 4300

India
t 91 124 4604000
f 91 124 4604099

Japan
t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6572-9740
f 65 6735-2913

Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE
CSEurope@corning.com

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 020 655 79 28
f 020 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries
t +31 (0) 206 59 60 51
f +31 (0) 206 59 76 73

LATIN AMERICA
grupoLA@corning.com

Brazil
t 55 (11) 3089-7400

Mexico
t (52-81) 8158-8400