protein immobilization

Coupling Using Sulfhydryls

SulfoLink® Coupling Gel

Many immobilizing supports couple through amino groups. The random coupling of antibodies through their amino groups could sterically hinder antigen binding capabilities. An antibody treated with 2-Mercaptoethylamine•HCl (Product #20408) will be reduced in the hinge region, yielding two fragments capable of binding antigen.[12] The two fragments formed are each composed of a heavy and light chain and contain free sulfhydryls (Figure 5). Pierce's SulfoLink® Coupling Gel (Product #'s 20401, 20402, 20403) is a 6% cross-linked beaded agarose support that is activated to allow covalent coupling of proteins through their sulfhydryl groups.[13,14] The activation chemistry results in a 12 atom spacer arm with a terminal iodoacetyl group that is preferentially reactive with sulfhydryl groups using appropriate coupling conditions (see Figure 6). Using SulfoLink® Coupling Gel to immobilize reduced antibodies through the free sulfhydryls will allow covalent coupling in the hinge region, theoretically leaving the antigen binding site unaffected.

SulfoLink® Coupling Gel is also useful for immobilizing a peptide with subsequent affinity purification of anti-peptide antibodies.[15] SulfoLink® Gel can be used to immobilize peptides that contain free sulfhydryls or are synthesized with a terminal cysteine residue. By coupling the terminal sulfhydryl groups to the SulfoLink® support, the specific anti-peptide antibodies can be pulled out—without absorbing out cross-reactive anti-carrier protein-antibodies. SulfoLink® Coupling Gel can also be used to immobilize many proteins. Any protein that contains a free sulfhydryl, or a disulfide bond that can be reduced, can be immobilized to SulfoLink® Gel. Table 8 demonstrates the coupling efficiency of various sulfhydryl containing proteins.

The SulfoLink® iodoacetyl chemistry is specific for free thiols. In addition, the iodoacetyl functional group assures an irreversible linkage. Other activated gels having pyridyl disulfide or dithio-5-nitrobenzoic acid moieties produce disulfide linkages that are cleavable between the ligand and the matrix.

Pierce's ImmunoPure® Ag/Ab Immobilization Kit #2 (Product #44895) is supplied complete with 5 x 2 ml prepacked columns of SulfoLink® Gel. This allows coupling of the ligand and chromatographic separation all in the same column. In addition, this kit includes all the buffers and reagents needed to complete the immobilization. An ImmunoPure® Ag/Ab Immobilization Trial Kit #2 (Product #20405) is also available and can be used to prepare one 2 ml column. SulfoLink® Coupling Gel is available in three package sizes—10 ml (Product #20401); 50 ml (Product #20402); and 500 ml (Product #20403).

Figure 5: Reduced IgG Molecule.

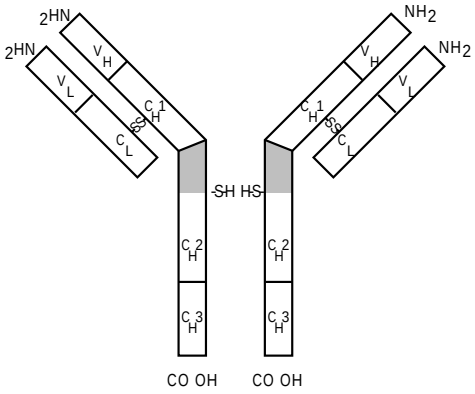


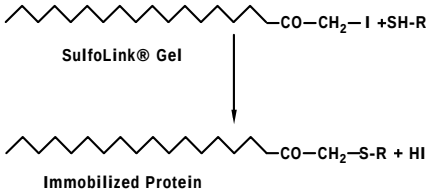
Table 8: Coupling of -SH/S-S Containing Proteins to SulfoLink® Coupling Gel Columns.

Protein	M.W.	-SH Groups/ Molecule	S-S Groups/ Molecule	Coupling Efficiency
Ceruloplasm	150,000	1 and 3	–	75%
Aldolase	147,000	7 and 28	–	87%
BSA	66,000	0.7	17	25%
HSA	66,000	0.7	17	72%
Ovalbumin	45,000	3 and 4	1	40%
B-Lactoglobin	36,000	2	2	63%
Trypsin	24,000	0	6	13%
Thioredoxin	11,700	2	1	20%
[Arg^8]Vasopressin	1,084	0	1	90%

A variety of proteins can be immobilized to the SulfoLink® matrix through -SH groups. 8 mg of all proteins were immobilized to 2 ml SulfoLink® Coupling Gel columns according to the coupling protocol. Two mg of the reduced form of the peptide, [Arg^8]Vasopressin, in 2 ml of SulfoLink® Coupling Buffer, was applied to a SulfoLink® column.

Note: Both BSA and Trypsin were turbid in the Incubation Buffer.

Figure 6



References

13. Gurd, F.R.N. (1967). Carboxymethylation. *Meth. Enzymol.* **11,** 532-541.
14. Metrione, R.M. (1982). Bromoacetyl Sepharose: a solid phase inhibitor of sulfhydryl enzymes. *Anal. Biochem.* **120,** 91-94.
15. Gentry, L.E., Rohrschneider, L.R., Casnellie, J.E. and Krebs, E.G. (1983). Antibodies to a defined region of pp60^{src} neutralize the tyrosine-specific kinase activity. *J. Biol. Chem.* **258,** 11219-11228.

protein immobilization

Immobilized TNB-Thiol

Immobilized TNB-Thiol (Product #20409) is a versatile affinity matrix designed to couple sulfhydryl-containing proteins or low molecular weight thiols. These proteins can be coupled under mild, non-denaturing reducing conditions. When sulfhydryl groups are not present, they may be generated by reducing disulfide bonds that are present. Alternatively, sulfhydryl groups may be introduced using Traut's Reagent (Product #26101). Traut's Reagent reacts with primary amines and leaves an exposed sulfhydryl group available for coupling. The cross-linked agarose support to which the TNB-Thiol is covalently coupled will withstand strong denaturants, low pH conditions and some solvent exposure.

In antigen or antibody immobilization applications, it is essential to keep the antigen binding site of the antibody available for binding to an antigen. TNB-Thiol will couple a reduced antibody in its hinge region, resulting in an immobilized antibody that is not sterically hindered at the antigen binding site (see Figure 7). Once you have immobilized your ligand (Figure 8a), the disulfide linkage can provide you with two means for recovering the antigen during affinity purification (Figure 8b). By using an acidic elution buffer, the researcher can elute only the antigen and reuse the column (Figure 8c). To avoid acidic elution conditions, a reducing agent such as DTT (Product #20290) can be used in the elution buffer. This will allow the removal of both the immobilized ligand and the antigen from the column (Figure 8d).

Low molecular weight thiols, peptides, immunoglobulins (Fab') and other proteins can be efficiently coupled to TNB-Thiol and then recovered by cleaving the disulfide bond with DTT. Less non-specifically bound protein or peptide would be eluted with the desired protein when using the thiol cleavable Immobilized TNB-Thiol compared to other supports that require low pH elution or a physical means of bond disruption. Immunoglobulins and Fab' fragments can be immobilized onto the support and used to bind antigen.[16] The antigen can then be recovered without denaturation from exposure to low pH conditions. This support may not work well in antibody/antigen applications in which the affinity between the two is weak, because there is only one antigen binding site available on a reduced antibody.

Figure 7: Reduced IgG Coupled to TNB-Thiol Agarose.

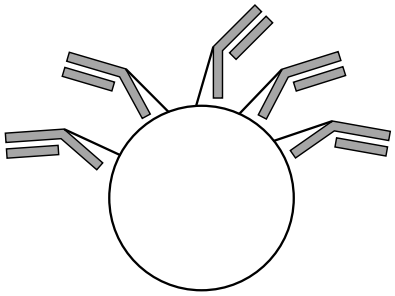


Figure 8a: Reduced IgG displaces TNB.

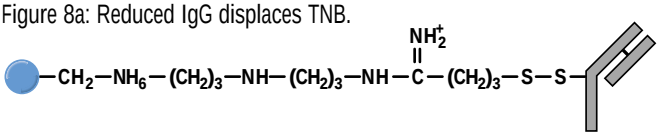


Figure 8b: Antigen binds to immobilized reduced IgG.

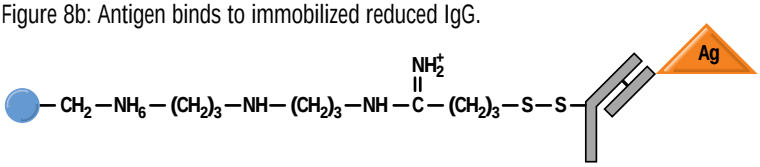
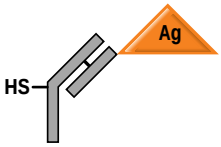


Figure 8c: Acid-Salt Elutes Antigen.

Figure 8d: DTT Elutes Antibody-Antigen Complex.



Immobilized *p*-Chloromercuribenzoate

Another alternative for coupling through sulfhydryls is to use Immobilized *p*-Chloromercuribenzoate (Product #20231). This affinity ligand binds sulfhydryl-containing proteins, as well as thiol derivatized DNA. The support is a cross-linked 6% beaded agarose with a ethylenediamine spacer.

Applications

- Purifying L-pyrrolidonecarboxylate peptidase (*B. amyloliquifaciens*),[17] wheat germ porphobilinogen deaminase[18] and L-glutamine D-fructose-5-phosphate amino-transferase[19]
- Isolating newly synthesized DNA of HeLa cells containing ß-2' deoxy-6-thioguanylate[20]

References

16. Jayabaskaran, C., Davison, P. and Paulus, H. (1987). Facile preparation and some applications of an affinity matrix with a cleavable connector arm containing a disulfide bond. *Preparative Biochemistry* **17(2),** 121-141.