

# Prepare molecules with poor solubility for immobilization on affinity supports

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#### Introduction

An excellent strategy for affinity purification and identification of research molecules, such as antibodies raised against a peptide antigen or receptor proteins that bind a ligand, is to covalently immobilize the peptide or ligand to a solid support. Thermo Scientific Pierce Protein Research Products include several agarose- and acrylamide-based resins that are preactivated to couple ligands through different specific functional groups, such as primary amines or sulfhydryls (see Related Products). Sodium phosphate or other simple buffers are recommended for the coupling reactions with these supports. However, hydrophobic proteins and certain peptides or other ligands may not be readily soluble in the recommended coupling buffers. This Tech Tip describes additives or alternative buffers that can be used in these circumstances.

Three issues must be considered when choosing a solubilization/coupling buffer with affinity supports:

- The effect of the buffer on the molecule to be coupled. That is, does the buffer denature or otherwise destroy the salient structural features of the study molecule?
- The effect of the buffer on the resin (beaded support). Does the buffer denature or destroy the resin?
- The effect on the coupling chemistry involved. Does the buffer contain chemical functional groups that compete with, block or otherwise quench the coupling reaction?

## AminoLink<sup>®</sup> and AminoLink Plus Coupling Resin

Immobilization to Thermo Scientific AminoLink Coupling Resin involves reaction to primary amino (-NH<sub>2</sub>) groups on the target molecule (e.g., side-chain of lysine or N-terminus in peptides and proteins) by reductive amination to aldehydes on the crosslinked beaded agarose support. The support itself will tolerate a wide range of water-miscible solvents including acetone, dioxane, alcohols, dimethylformamide (DMF) and dimethylsulfoxide (DMSO) if the exchange into these solvents is made in a stepwise gradual manner. Because of the reaction chemistry, solvent components must be devoid of primary amines and oxidants.

Molecules that cannot be dissolved directly in the recommended sodium phosphate or carbonate/citrate buffer may be soluble in one of the agarose-compatible water-miscible solvents mentioned above. If dissolved first in one of these solvents (e.g., DMSO), the molecule may remain soluble when added to the coupling buffer at a final solvent concentration of 10-30%.

Chaotropes, which are substances that disrupt the structure of water interactions, may help to solubilize hydrophobic molecules for coupling to AminoLink Resins. For example, hydrophobic peptides and proteins often can be dissolved in coupling buffer containing either 6 M Guanidine•HCl (Product No. 24110) or 4 M Urea (Product No. 29700). However, because the degradation products of guanidine and urea include ammonia, which contains a primary amine that will compete for reaction to the support, urea and guanidine solutions must be prepared with quality reagents immediately before use.

In addition to increasing solubility of hydrophobic molecules, guanidine and urea are general protein denaturants, unfolding proteins and altering their three-dimensional structures. Consequently, some proteins will be irreversibly altered upon action of these compounds and may lose their binding function. Before large-scale immobilization of those macromolecules that must be dissolved with guanidine or urea, researchers should test a small sample to determine whether these denaturing effects will adversely affect affinity purification.

Finally, proteins are not particularly soluble when the buffer pH is the same as the protein's isoelectric point (pI). If this is the case, adjustments to the default coupling buffers may be made. Immobilization to AminoLink Resin is successful in amine-free buffers between pH 6.5 and 10. Indeed both regular (pH 7.2) and enhanced (pH 10) coupling buffers are described for use with AminoLink Plus Coupling Resin.



## UltraLink<sup>®</sup> Biosupport

Thermo Scientific UltraLink Biosupport is a unique affinity support prepared by co-polymerization of azlactone and bisacrylamide. The durable resin couples to primary amino groups at pH 4-9. Like beaded agarose, UltraLink Biosupport is compatible with a variety of water-miscible solvents, including DMF and DMSO. Many buffers that are devoid of amines and sulfhydryls may be used effectively for coupling. Because the reactive azlactone group is somewhat hydrophobic, coupling of the target amino groups in most soluble proteins and ligands is more efficient when the reaction buffer includes lyotropes, which are substances that promote the structure of water interactions. The product instructions recommend using 0.6 M sodium citrate or 0.8-1.5 M sodium sulfate as lyotropes in the coupling buffer.

Hydrophobic molecules, of course, will not require lyotropic agents to promote contact with the reactive groups and in fact will not be soluble in buffers containing these agents. For hydrophobic molecules, DMF or DMSO or chaotropes like urea and guanidine•HCl may be useful (as described for AminoLink Resin in the previous section).

Choosing a coupling buffer of different pH than the isoelectric point of protein will aid in solubilization. For this purpose, adding 1 N NaOH, 1 N NH<sub>4</sub>OH or 0.1 N phosphoric acid to the coupling buffer may be effective. In general, alkaline conditions are more favorable for rapid and efficient coupling, but slightly acidic conditions also can be effective.

The instructions for UltraLink Biosupport recommend using 0.1% Triton X-100 to aid in "wetting" and hydrating the dried beads before use; this or other detergents may also aid in solubilizing hydrophobic proteins. A higher concentration of detergent (as might exist in a membrane protein extract) also may be compatible with the coupling reaction, although this has not been tested by our researchers.

# SulfoLink<sup>®</sup> Coupling Resin and UltraLink Iodoacetyl Resin

Thermo Scientific SulfoLink Coupling Resin and UltraLink Iodoacetyl Resin allow covalent immobilization of molecules containing sulfhydryl (-SH) groups in alkaline buffers. SulfoLink Coupling Resin is a crosslinked beaded agarose support; therefore, the same water-miscible solvents discussed for the AminoLink Resins are compatible with this activated support. UltraLink Iodoacetyl Resin is a derivatized form of UltraLink Biosupport. Because the iodoacetyl-modified azlactone group is not hydrophobic like the nonderivatized group (discussed in the previous section), lyotropic agents have no particular benefit in promoting immobilization to UltraLink Iodoacetyl Resin.

DMSO and other water-miscible solvents are compatible in the coupling reaction at final concentrations up to 20%. Higher concentrations of solvent may be compatible, but this has not been confirmed. Among chaotropes, guanidine•HCl is recommended over urea because cyanates are a common degradation product of urea, and these can react with sulfhydryls. A final concentration of 4 M guanidine•HCl is compatible in the coupling reaction.

Coupling buffer pH may be modified only slightly for coupling to iodoacetyl-activated supports because the reaction depends on the degree of ionization of the target functional group. At high pH, undesirable reaction to amino groups can occur. In acidic conditions, reaction to the target sulfhydryls is poor. Therefore, immobilization of sulfhydryl-containing molecules to iodoacetyl resins is best performed in mildly alkaline conditions (pH 7.5-9.0).

### **Related Thermo Scientific Products**

44894	AminoLink Plus Immobilization Kit, $5 \times 2$ ml columns and reagents
53110	UltraLink Biosupport, 1.25 g (makes 8-10 ml)
44995	SulfoLink Immobilization Kit for Proteins, $5 \times 2$ ml columns and reagents
44999	SulfoLink Immobilization Kit for Peptides, $5 \times 2$ ml columns and reagents
53155	UltraLink Iodoacetyl Resin, 10 ml
44910	CarboLink <sup>TM</sup> Immobilization Kit, 5 × 2 ml columns and reagents
53149	UltraLink Hydrazide Resin, 10 ml
44899	CarboxyLink <sup>TM</sup> Immobilization Kit, 5 × 2 ml columns and reagents

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.

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