protein immobilization

Coupling Using Carbonyls

CarboLink® Coupling Gel (Product #'s 20391, 20392, 20392) allows immobilization of glycoproteins through their oxidized carbohydrate moieties. Because carbohydrates are located on the Fc portion of antibody molecules, CarboLink® Gel has the advantage of orienting the antibody molecule by binding to the Fc portion. This allows the antibody binding sites to remain unobstructed, resulting in greater purification capability.

The CarboLink® Gel may be used for a variety of glycoproteins. The coupling efficiency of antibodies and other glycoproteins depends on the amount and accessibility of carbohydrate moieties. Eight randomly selected glycoproteins yielded an average coupling efficiency of 79% (Table 9).

The immobilization chemistry uses sodium periodate to oxidize glycoproteins, converting vicinal hydroxyl groups to reactive aldehyde groups, which then react with hydrazide groups on the matrix to form hydrazone bonds (Figure 9). This support also offers minimal protein leakage and good coupling. Because no reductant is used, and the reaction is performed at neutral pH, coupling conditions are mild. This is useful when sensitive antibodies are being used. The coupling conditions are also flexible with regard to time and temperature. Minimal non-specific binding and a long 23 atom spacer arm make this a favorable support for affinity chromatography. The protein coupled columns may also be regenerated at least ten times when following the protocol given in the instruction booklet.

CarboLink® Coupling Gel is also available in two kit formats—the ImmunoPure® Ag/Ab Immobilization Kit #3 (Product #44900) and the ImmunoPure® Ag/Ab Immobilization Trial Kit #3 (Product #20390). The trial kit contains sufficient reagents and buffers for preparing one 2 ml column, while the larger kit can be used to prepare five 2 ml columns. The CarboLink® Gel provides an immobilization technique that has rapid, reproducible coupling to carbohydrate or *cis*-diol moieties. The gel has a long shelf life, and the covalent bond formed between the protein and the matrix is stable, resulting in excellent purification capabilities.

Table 9: Coupling Efficiency of Glycoproteins to CarboLink® Gel.

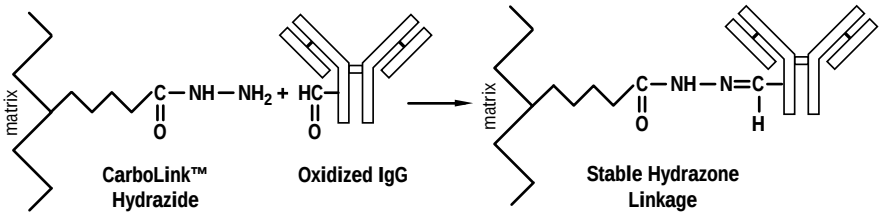


Figure 9: Attachment of Oxidized IgG to CarboLink® Coupling Gel.

Protein	M.W.	Coupling Efficiency
Collagen (Type VI)	163,000	63%
Human IgG	150,000	74%
Avidin	66,000	95%
Chorionic Gonadotropin	59,000	91%
Fetuin	48,700	87%
Ovalbumin	45,000	86%
α_1-Acid Glycoprotein	44,100	90%
Pepsin	34,700	47%

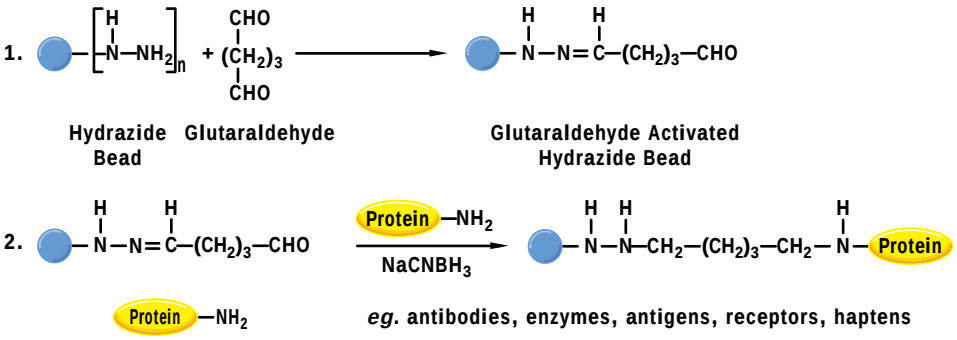
A variety of glycoproteins can be immobilized to CarboLink™ Gel through their oxidized carbohydrate moieties. Two mg of protein were added to 2 ml of CarboLink™ Gel and immobilized according to the standard coupling protocol.

protein immobilization

Hydrazide Beads

An alternative for coupling to hydrazide functional groups is to use Pierce's Hydrazide Beads (Product #20202). These beads are hydrazide derivatized, uniform, non-porous spherical polystyrene beads. These beads may be activated with glutaraldehyde and then used to couple antibodies to hydrazide functional groups (Figure 10). An alternative coupling method has been reported by O'Shannessy, *et al.* [21] This method couples antibodies through carbohydrate groups in the Fc region, resulting in site-directed immobilization. Unlike the glutaraldehyde method shown in Figure 10, the O'Shannessy method has a one-step coupling protocol for oxidized IgG. The chemistry is quite similar to that of CarboLink® Coupling Gel.

Figure 10: Hydrazide Beads.



References

21. O'Shannessy, D.J. and Hofmann, W.L. (1987). Biotech. *Appl. Biochem.* **9,** 488-496.