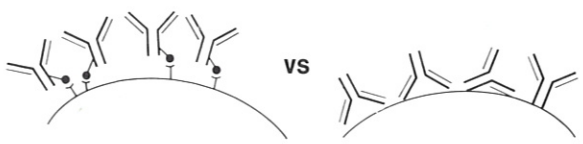
BIO-RAD

US/EG

Bulletin 1424

Affi-Gel® Hz Immunoaffinity Kit

The Affi-Gel Hz immunoaffinity kit achieves a more optimal orientation of coupled antibody than currently available activated supports which couple via primary amines. The kit contains hydrazide activated agarose beads which are used to couple IgG molecules via carbohydrate moieties located on the Fc region of the antibody. Fc attachment results in a higher antigen capacity than can be achieved with conventional supports. With conventional matrices which couple antibodies randomly via primary amine or carboxyl groups, antibodies may be attached at or near the binding site, resulting in loss of capacity.



Oriented coupling results in greater antigen binding capacity per coupled IgG. IgG immobilized on Affi-Gel Hz hydrazide gel through the carbohydrate of the Fc region.

Random coupling yields low antigen binding due to attachment at or near the binding site of the antibody. IgG coupled directly to agarose support via primary amine.

Carbohydrate moieties on Fc region of IgG.

Hydrazide functional group on gel spacer arm.

Immunoglobulin G.

Coupling Mechanism

Immunoglobulins are glycoproteins which contains carbohydrate located on the Fc region of the antibody. Periodate oxidation of vicinal hydroxyl groups of the carbohydrate results in the formation of aldehydes for specific coupling to Affi-Gel Hz hydrazide gel. The result is a stable, covalent hydrazone bond linkage that is chemically resistant to many common elution conditions employed in affinity chromatography. This stable bond, along with the oriented coupling offered by the support, results in superior performance in immunoaffinity chromatography.

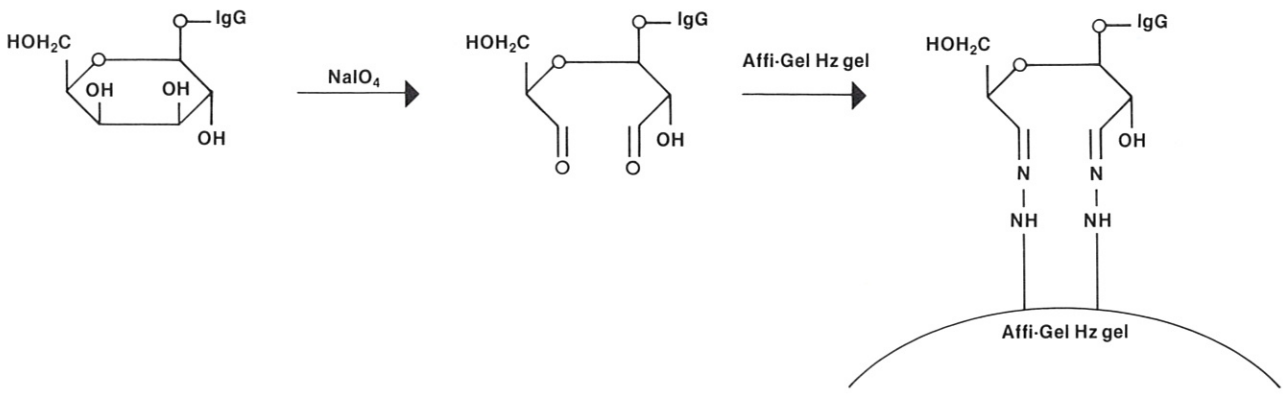
Increased Efficiency in Immunoaffinity Chromatography

Immunoaffinity chromatography exploits bio-specific antigen-antibody interactions to achieve a high degree of purification in a single step. With the Affi-Gel Hz immunoaffinity kit, column capacity can be improved and a high degree of stability maintained. The Affi-Gel Hz immunoaffinity kit contains everything necessary to couple IgG, IgM and other Immunoglobulin classes, to the low pressure agarose support for immunoaffinity chromatography applications.

Antigen Binding Capacity of Immobilized IgG

Studies comparing the antigen binding capacity of immunoaffinity matrices demonstrate the superior performance of Affi-Gel Hz hydrazide gel over CNBr-activated Sepharose® gel.

Hydrazide Coupling Chemistry



Sugar residue of carbohydrate on the Fc region of IgG.

Periodate oxidation of vicinal hydroxyls to form aldehydes.

Oxidized IgG specific coupling to Affi-Gel Hz hydrazide gel.

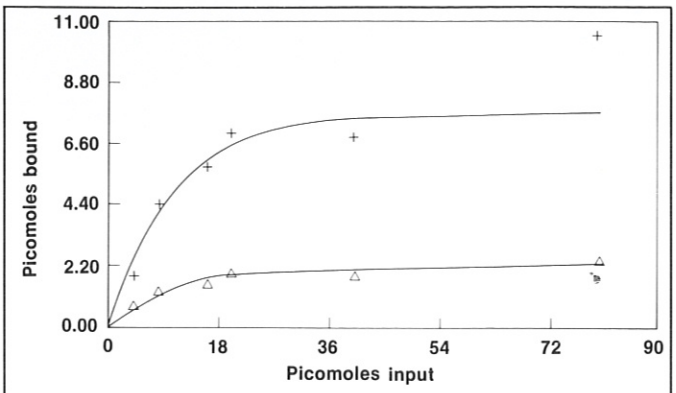


Fig. 1. Binding of ^{14}C-BSA to anti-BSA IgG immobilized on Affi-Gel Hz (+) and Sepharose gel (Δ).

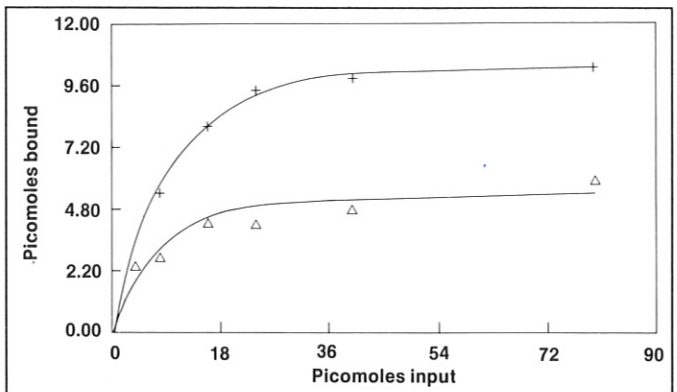


Fig. 2. Binding of ^{14}C-beta-galactosidase to anti-beta galactosidase IgG immobilized on Affi-Gel Hz (+) and Sepharose gel (Δ).

Figure 1 shows that Affi-Gel Hz gel has a 300% higher antigen binding capacity than CNBr-activated Sepharose gel-IgG. Oxidized IgG specific for bovine serum albumin was coupled overnight at room temperature to Affi-Gel Hz gel or for 2 hours at room temperature to CNBr-activated Sepharose gel according to the manufacturers instructions. Both supports were washed with 10 mM phosphate, 0.5 M NaCl, pH 7.0, and gel volumes representing equivalent amounts of IgG were incubated overnight at room temperature with increasing quantities of ^{14}C-BSA. The gels were washed several times, and the bound BSA was determined by liquid scintillation counting.

Figure 2 demonstrates a 200% increase in beta-galactosidase capacity for Affi-Gel Hz gel coupled IgG affinity support over the same antibody immobilized on CNBr-activated Sepharose gel. ^{14}C labeled beta-galactosidase was used to determine the binding capacity of each support and the conditions and methods were as described for Figure 1.

Immobilization Procedure

IgG to be immobilized for affinity purification should be of high purity to maximize column capacity and effectiveness.

1. Purified IgG is exchanged into diluted coupling buffer pH 5.5 using an Econo-Pac® 10DG column.
2. The IgG is oxidized with sodium periodate for 1 hour at room temperature, light protected. This mild oxidation of vicinal hydroxyls of sugars on the carbohydrates is specific and does not alter IgG activity (data not shown).
3. Immediately after oxidation, any unreacted periodate is removed by desalting on an Econo-Pac 10DG column.
4. The oxidized antibody is allowed to couple overnight at room temperature.
5. The Affi-Gel Hz gel immobilized IgG is poured into the 1 × 10 cm Econo-Column® chromatography column provided, washed and ready for affinity purification.

Scale-Up Applications

Scale-up quantities of gel, oxidizer, and 10× buffer are available for preparative immunoaffinity purification. Large scale purification using Affi-Gel Hz hydrazide gel for specific IgG coupling extends the advantages in coupling chemistry from the kit to preparative applications.

Ordering Information

Catalog Number	Product Description
153-6060	**Affi-Gel Hz Immunoaffinity Kit,** includes Affi-Gel Hz hydrazide gel, 5 ml in isopropanol; Affi-Gel Hz oxidizer, 25 mg sodium periodate; Affi-Gel Hz 10 × coupling buffer concentrate, 25 ml; Econo-Pac 10DG desalting columns, 2; 1 × 10 cm Econo-Column, 1; instruction manual.
153-6047	**Affi-Gel Hz Hydrazide Gel,** 25 ml
153-6048	**Affi-Gel Hz Hydrazide Gel,** 4 × 25 ml
153-6049	**Affi-Gel Hz Hydrazide Gel,** 500 ml
153-6054	**Affi-Gel Hz 10 × Coupling Buffer,** 500 ml
153-6055	**Affi-Gel Hz Oxidizer,** 250 mg sodium periodate
732-2010	**Econo-Pac 10DG Desalting Columns,** 30
732-8102	**Luer-Lock 2-Way Stopcocks,** 10

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