Generation of Protein Ligand Affinity Columns That Reduce Protein Leaching

Proteomics research has enabled researchers to identify new proteins, novel interactions and new binding partners and protein complexes. A major tool in the identification of new proteins and novel interacting proteins are affinity resins. These resins have a particular ligand (e.g. a protein, antibody or peptide) chemically linked or coupled to a solid support, such as agarose. A biological sample is passed over the column and proteins that interact with the ligand bind to the column and non-interacting proteins are washed away. The bound proteins are then eluted and further proteomic techniques are used to identify novel interacting proteins.

We have developed two activated resin kits for the generation of affinity resins and columns.

*The **amine reactive HOOK™ Agarose Coupling Kit** is designed to bind to exposed primary amines on proteins and peptides (Figure 1). This kit is ideal for immobilizing all proteins and peptides, due to the interaction with exposed primary amines, with minimal leaching of the proteins and peptides. The kit uses a reactive glyoxal group that is coupled to 6% agarose beads. The aldehyde groups on the reactive glyoxal moiety will react with the ligands containing primary amine groups to form intermediate Schiff Base complexes. These intermediate Schiff Bases are stabilized by the addition of sodium cyanoborohydride, which will only reduce the Schiff Base intermediate and, unlike sodium borohydride, will not reduce the aldehyde groups. The resultant ligands are far more stable and less prone to leaching than those coupled using the typical reducing agent cyanogen bromide.*

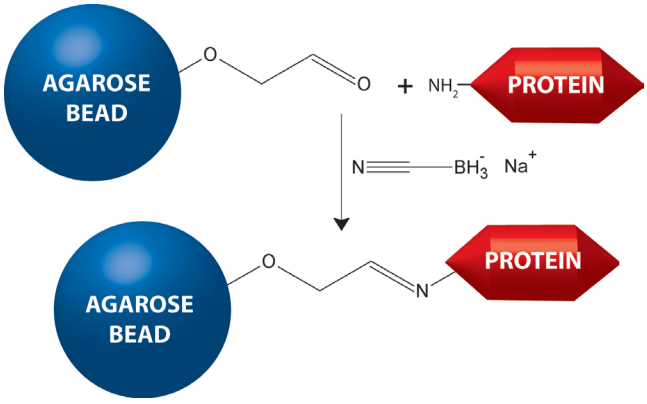

Figure 1: Schematic of ligand binding to reactive glyoxal group on agarose beads

*The **Sulfhydryl Immobilization Kit for Proteins** is designed for the simple and efficient coupling of proteins to a solid agarose support. The Sulfhydryl Coupling Resin Columns utilizes iodoacetyl groups that specifically react with free sulfhydryls to form covalent, permanent thioether bonds (see figure 2). The long spacer arm reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification. The specificity for sulfhydryl groups allows better orientation of proteins and peptides on the agarose, due to the known and/or engineered position of sulfhydryl residues.*

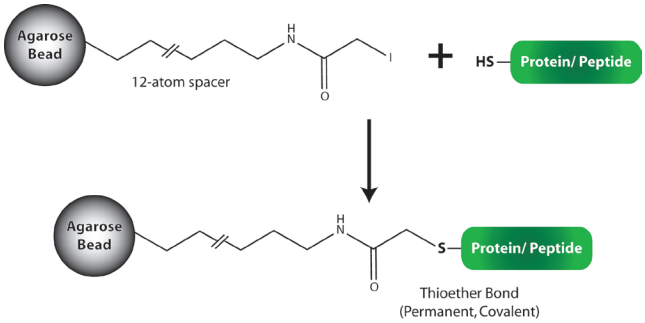

Figure 2: Schematic of Ligand binding to reactive iodoacetyl group on agarose beads.

AIM

To evaluate the amine and sulfhydryle reactive agaroses by following the binding of a ligand to the resins.

METHODS

HOOK™ *Agarose Coupling Kit (Amine)*

2ml HOOK™ glyoxal activated agarose was resuspended and equilibrated in our high pH proprietary reaction specific Optimizer Buffer™-VI. The high pH of the Optimizer Buffer™-VI will generate a greater yield of Schiff Base formation at a greater rate. In addition, Optimizer Buffer™-VI contains no amines, such as Tris or glycine, that could potentially inhibit the coupling reaction. Once fully equilibrated, 60mg protein was added at a concentration of 30mg/ml, followed by 200µl 0.1M sodium cyanoborohydride to reduce the Schiff base to a stable secondary amine.

Sodium cyanoborohydride was used, as opposed to the more commonly used cyanogen bromide, as it is specific for the reduction of the Schiff base intermediate. Cyanogen bromide reduces both the Schiff base intermediate and the free aldehyde groups and therefore has an inhibitory effect.

Following a six-hour incubation at room temperature,

the protein concentration of the unbound material was compared to the starting material and the binding efficiency calculated. The protein-coupled agarose was washed and the unreacted aldehyde groups were inhibited with the addition of the blocking agent, 5ml ethanolamine (12mg/ml). The new stable affinity column was stored at 4°C in PBS supplemented with 0.05% sodium azide.

HOOK™ *Agarose Coupling Kit (Sulfhydryl)*

2ml Sulfhydryl Coupling Resin was resuspended and equilibrated in our proprietary reaction specific Optimizer Buffer™-II. 15mg protien was reduced with the supplied 2-mercaptoethylamine at 37°C for 90 minutes. After reduction the reducing agent was removed by passage through a supplied SpinOUT™ GT-600 desalting column. The reduced protein was added to the equilibrated agarose-suspension and incubated for 30 minutes at room temperature.

Following incubation, the protein concentration of the unbound material was compared to the starting material and the binding efficiency calculated. The protein-coupled agarose was washed and the unreacted maleimide groups were inhibited with the addition of the blocking agent, L-Cysteine • HCl. The new stable affinity column was stored at 4°C in PBS supplemented with 0.05% sodium azide.

RESULTS AND DISCUSSION

The binding affinity of the resins was calculated by comparing the protein concentration of the original protein sample with that of the flow through. The protein concentration was determined by using G-Biosciences' *NI*™-Protein Assay. The graph in figure 3 shows the percentage binding of the different resins and figure 4 depicts SDS-PAGE analysis of the starting and flow through materials.

The Sulfhydryl Immobilization Kit for Proteins bound ~96% of the starting material, so has a coupling efficiency of ~2.7mg protein/ml. The amine reactive HOOK™ Agarose Coupling Kit bound ~94.6% of the starting material, so has a coupling efficiency of ~16.6mg protein/ml.

Both HOOK™ Agarose Coupling Kits can be utilized in the generation of stable affinity binding columns for your protein or peptide of interest. Coupling was greatly increased with the use of reaction specific Optimizer Buffer™, which does not affect downstream reactions.

REFERENCES

HOOK™ Activated Agarose (Amine Reactive)
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NI™ (Non-Interfering™) Protein Assay
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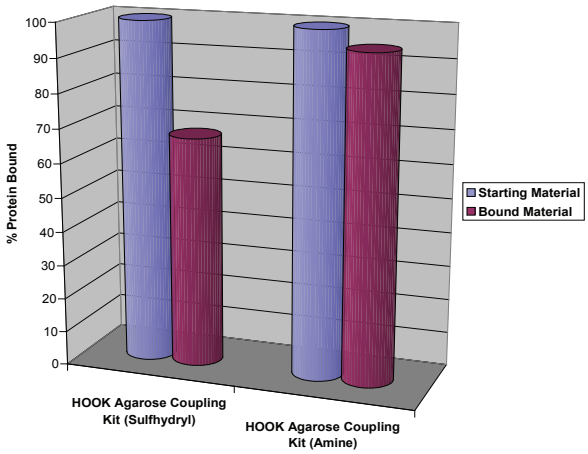

Figure 3: Graph showing the coupling efficient of the HOOK™ Agarose resins.

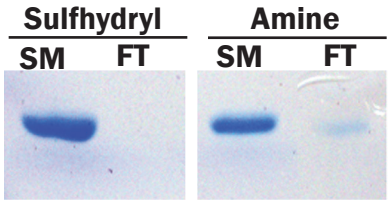

Figure 4: SDS polyacrylamide gel depicting coupling efficiency of the agarose resins. Proportional volumes of starting material (SM) and flow through (FT) resolved on a 4-20% gradient gel, staine with RAPID*stain*™.

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ORDERING INFORMATION

Cat. No.	Description/ Size
786-063	HOOK™ Agarose Coupling Kit (Amine Reactive)/ 5 columns
786-064	HOOK™ Agarose Coupling Kit (Sulfhydryl Reactive)/ 5 columns
786-005	*NI*™ Protein Assay Kit/ 500 assays
786-31	RAPID*stain*™/ 1 liter

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