mixed every 15-20 min for 5-10 seconds. After the incubation, let the tube stand upright for 15-30 min without shaking. Completely drain the protein solution by opening the bottom cap. It can be saved to measure A280 to determine the protein coupling efficiency.

4. Block the non-specific binding sites by adding **2**x the gel volume (if gel volume after rehydration is 5 ml then 2x-gel= 10 ml) of the buffer # 2. Replace the bottom and top cap. Mix it manually for 5-10 seconds and allow it to drain completely.
5. Wash with 5 bed volume of **buffer #3**.
6. Wash with 5 bed volume of **buffer #4**.
7. Equilibrate the column by passing 5x-gel bed volume of buffer #2. Replace the bottom cap and add 5-10 ml of buffer # 2. Add sodium azide (0.1% w/v if desired). Store the affinity column at 4oC or proceed with antibody purification using **General protocol of affinity purification** (request a protocol from ADI if needed). Do not freeze the affinity gel.

ADI also has an **Antibody purification kit** containing all buffers (binding, wash buffer, elution buffer, neutralization buffers sufficient for 10-20 antibody purifications; cat # 110300-AF).

The peptide or protein is now coupled to the gel. Typically, affinity column can be stored at 4oC for up to 3-6 month or longer depending upon the stability of the coupled peptide or proteins. Most affinity column can be used multiple times 3-10 times. Eventually, Agarose coupled peptide or protein will leak off the column slowly, particularly when exposed to multiple purification protocol, or become inactivated due to oxidation or modification of susceptible amino acids or functional groups on the proteins. Therefore, stability of the column must be evaluated for a given peptide or protein.

It is important to note that covalent coupling of the peptides or proteins may change the conformation and the biological property. It may show a reduced or no biological activity or antibody-antigen activity. There is no way to predict this based upon the peptide structure or protein.

Most affinity purification protocol for antibody purification use either a low (pH 2-3) or high pH buffers (pH 10-12) or Chaotropic salts such as Guanidine-Hcl or Urea for antibody elution. These agents may affect the antibody activity.

Trouble shooting and Common Problems

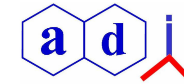
1. Peptide/protein precipitates in coupling buffer-Many peptides and proteins are not soluble in aqueous buffer. It may help to add up to 20% DMSO or 4M urea to improve solubility. The solubility of the peptides must be confirmed in the coupling buffer before using it on N-link.
2. Low coupling efficiency could be due to the absence of free NH2 groups.
3. Loss or no binding of the antibodies on the affinity column-Many peptides or proteins will change conformation upon covalent linkage and may lose it biological property or antibody binding ability. This is not due to the affinity matrix. A new coupling method, using a different coupling chemistry such as Cysteine linkage that utilizes free Cysteine groups (#110100-CG, Cys-Link gel), should be tried.
4. Bound antibody will not elute or lose antigen binding ability after elution-Due to changes in the peptide conformation after linkage, the affinity support may only bind certain type of antibodies that are not functional or have low titer. Many antibodies will lose the activity when eluted under low or high pH. This is also not related to N-link Agarose but the inherent property of the peptide or antibodies under investigation. It may help to test other peptide linking protocols or use various elution buffers.

Instruction Manual 110200-NG

Preactivated N-Link-Affinity Gel Agarose

Cat. # 110200-NG

For covalent coupling of NH2 containing peptide and proteins to Agarose



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Preactivated N-Link-Affinity Gel Agarose # 110200-NG

Kit Contents

1. **Preactivated N-link gel powder 2 g #110200-1**: (1 g dry powder swells to 2.-3 ml gel volume upon activation) Supplied in stabilizing buffers in powder form. Use in required amounts. Store powder at 4oC.
2. 20-ml affinity column # AC-20
3. Coupling buffer (10X), 5 ml, # CB-10

Binding capacity: ~ 5-10 mg of IgG or 2-5 mg of synthetic peptide containing free amines per g.

Coupling time : ~2 hrs

Form and storage: Upon receipt store in a cool and dark place at 4oC.

Introduction

Antibodies to proteins are often made using short synthetic peptides (~20-25 amino acids). The antigenic peptides must be covalently coupled to the carrier proteins such as KLH or BSA using the free amino, carboxyl or free cysteine. Typically, the peptide or protein are also coupled to the agarose gels using the same functional group (Free Nh2, -COOH or cysteine) to prepare affinity gels. However, the process of agarose activation requires many chemicals and optimization of coupling reagents. Therefore, there is a loss of time, money, and inconsistencies in the process. ADI's N-Link Agarose is 4% **preactivated** beaded agarose that is supplied in stabilizing powder. It requires simples mixing with the target antigen or peptide to make the affinity column.

N-link agarose is made attaching 6-aminohexanoic acid to cyanogen bromide (CNBR) activated agarose. The NH2 group of the 6-aminohexanoic acid is attached to the resin. The carboxylic acid functional group is then converted to the NHS ester. This resin is specific for primary amino groups, so other active groups in the ligand do not need to be protected prior to coupling. When a compound with a primary amino group is coupled to the resin, the spacer arm between the compound and the resin is 8 atoms. N-link Agarose is suited for coupling antigen (peptides or proteins) or antibody.

Note: Peptide or proteins must not contain any buffer that may contribute free amines (e.g, Tris buffer must be removed).

I gram of N-link powder is sufficient for 2-4 mg of peptides or proteins.

Keep the N-link gel in powder form and use as needed.

Additional material required but not supplied

- Cold distilled or deionized water, dialyses abg
- Pipettes Tubes, beakers
- Buffer #1- 1M Tris, pH 7.4 (prepare at least 100 ml)
- Buffer #2- Dilute buffer #1 (1 ml) with water (100 ml) –prepare 200 ml
- Buffer #3- 0.1M Glycine-Hcl, pH 2.5 (prepare 25 ml)
- Buffer #4- 1.0M NaCl, Prepare 100 ml
- Buffer #5- 0.1M NaHCO3, pH 9.5 (500-1000 ml may be needed if protein solution is to be dialyzed).

Procedure for Coupling NH2-containing peptides or proteins

The peptide or protein to be coupled must contain free amines. Most peptides will have at least 1 Free NH2-group at the N-terminus unless it has been blocked or modified (N-terminal acetylation or addition of other groups). Additional amines may be contributed by lysines or other amine-containing amino acids in the peptide or proteins. The peptide or protein must also be soluble at pH 9-9.5 for N-Link gel.

1. Peptide or protein preparation for coupling to N-link gel

Peptide preparation- Dissolve the peptide powder in water first (2-5 mg peptide at 1 mg/ml). If the peptide do not dissolve in water then DMSO or DMF (solvents up to 20%) can be used. It is also possible to dissolve the peptides by the addition of dilute acid or base. Water soluble peptides can be directly dissolved in 1X coupling buffer. If the peptide is in water or required acid or base or DMF/DMSO then add 1/10th the volume of 10X coupling buffer (add 100 ul of 10x buffer per ml of the peptide solution). It is important to keep the pH of the peptide or protein ~9.0-9.5 (adjust the pH if necessary by adding 0.1N NaOH). Peptide solution should be within 1-2x the gel volume (e.g. if gel volume is 5 ml then the peptide solution should be 5-10 ml).

Protein preparation – Dissolve the protein at 0.5-1 mg/ml in 1x coupling buffer. If protein is in another buffer then it must be dialyzed extensively (500 mls, 4-6 hrs at 4oC or overnight) against the 0.1M NaHCO3, pH 9.5. Alternatively, the buffer can be exchanged using a desalting column and then adding the 1x coupling buffer. If desired, small sample can be saved for A280 or protein measurement before coupling.

2. N-link Agarose preparation

Transfer the required amount of powder gel (2 g is sufficient to make good size affinity column of 5-6 ml) in a 50-ml tube. Add 45-50 ml pre-chilled water. Gently rotate the tube end-to-end for 1-2 min to hydrate the gel. Let the tube stand for 5-10 minute at room temp for complete hydration. Transfer the gel suspension to the supplied 20-ml column and break the bottom cap to allow the water to drain in another tube or beaker. After draining the water, wash with another 200-300 mls of pre-chilled water and allow it to drain completely. Close the column by putting the supplied bottom cap or Parafilm. Add 2 ml of 1x coupling buffer to the gel. The gel should be used immediately for coupling peptide or proteins.

3. Peptide or protein coupling to N-Link gel.

Add peptide or protein solution (volume should be 1-2x the gel volume) to the column and gently mix it for 5-10 seconds. Incubate the tube for 1 hr at room temp or 4 hrs at 4oC on an orbital shaker or end-to-end mixer. Do not use a magnetic stirrer. If a shaker is not available, the tube can be manually