6. Drain the column or transfer the tube contents to the column and drain the solution. Save an aliquot to determine A280 or protein concentration to determine coupling efficiency.
7. **Block the non-specific binding sites using** Cysteine-Add 1 gel-bed volume to the column (e.g., to 5 ml gel add 5 ml blocking solution). Replace the bottom and top cap. Mix it manually for 5-10 seconds and allow it to stand for 30 min at room temperature without mixing.
8. **Wash the column**- Drain the blocking solution and wash with 5 bed volume of wash buffer (e.g., for 5-ml gel add 25-30 ml wash buffer).
9. **Equilibrate the column**-Wash with 2-gel bed volume (e.g., for 5-ml gel add 10 ml of storage buffer). 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 GSI if needed).

GSI 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 Cys-link.
2. Low coupling efficiency could be due to the absence of Sulfydryl or oxidized Cysteines. It may be necessary to reduce the peptides with DTT and removing the excess before coupling.
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 is the solution. It may help to test another coupling method that utilized free amino groups (#110200-NG, N-Link gel).
4. Bound antibody will note elute or lose antigen binding 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 low titer. Many antibodies will lose the activity when eluted under low or high pH. This is also not related to Cys-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 EW-110100-CG

Pre-activated Cys-Link-Agarose

KIT Cat. # EW-110100-CG

For covalent coupling of cysteine containing peptide and proteins to Agarose

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Preactivated Cys-Link-Affinity Gel Agarose # EW-110100-CG

Contents

1. **Cys-link gels Size**: 5 ml packed gel volume in stabilizing buffer supplied as 50% gel suspension in 10 mM EDTA-Na, 0.05% NaN3, 50% glycerol) in a brown bottle. Use in required volume (1-5 ml) at a time and store the rest at 4oC in supplied buffer.
2. A 10-ml affinity column
3. Cysteine buffer powder, 1 vial, dissolve in 10 ml of conjugation buffer

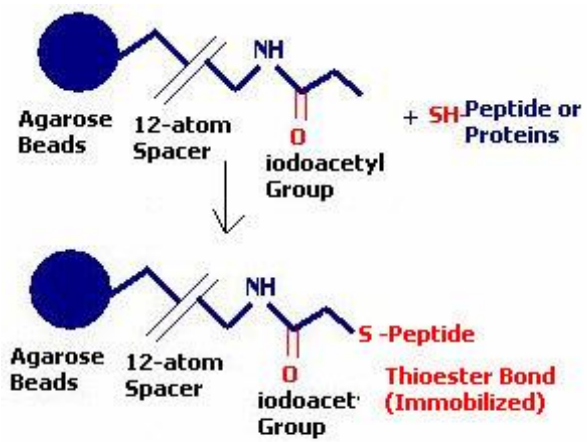
Binding capacity: ~ 5 mg of IgG or 1 mg of synthetic peptide containing free Cysteines per ml of packed gel.

Coupling time : 60 min

Form and storage: supplied in ready-to-use 10-ml affinity column. 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. Since cysteine (-Cys or free –SH group) are less abundant than the available –NH2 or –COOH in the antigenic peptides, coupling of peptide to the carrier proteins provides a single point attachment. A cysteine can be added, if not already present in the peptide, at the amino or the carboxyl terminus. The same cysteine can be utilized to couple peptides to commonly used solid support affinity gels such as Agarose or Sepharose. 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. Cys-Link Agarose or Sepharose is 4% **preactivated** beaded agarose that is supplied in stabilizing buffer. It requires simples mixing with the target antigen or peptide to make the affinity column.



Cys-Link Agarose (cat # EW-110100-CG) contains active Iodoacetyl groups that react specifically with free sulfydryl (-SH) groups on the peptides or proteins. Cys-Link Agarose is prepared with 12-atom carbon spacer to minimize steric hindrance of the coupled peptide or antigen. Cys-link Agarose is suited for purifying the antibody or any protein that can interact with the coupled ligand or peptide or antigen.

Additional material required but not supplied

- **Coupling buffer**- 50 mM Tris, 5 mM EDTA-Na, pH 8.5. Need approx. 20-times the volume of Cys-link gel support or ~200 mls.
- **Blocking buffer**-Dissolve the supplied Cysteine powder in 10 ml of coupling buffer.
- **Wash buffer**-1 M NaCl
- **Storage buffer**-PBS, pH 7.5 with or without azide (0.1% w/v) as preservative.

A **Cys-Link buffer kit** containing the coupling buffer, blocking buffer, wash buffer, and storage buffer can be obtained from GSI (cat # EW-110400-CB). The kit is sufficient for coupling 5-10 different peptide or proteins.

Procedure for Coupling Cys-containing peptides or proteins

The peptide or protein to be coupled must contain free (reduced) sulfydryl or Cysteines. If necessary, the ligand can be reduced with Dithiothreitiol (DTT) followed desalting or dialyses to remove the excess DTT before using it on Cys-link gel. Synthetic peptide containing free Cysteine can be directly used.

1. **Cys-link Agarose preparation**-Stir end-to-end or swirl the Cys-link to evenly suspend the supplied agarose. Equilibrate the required volume of Cys-link Agarose at room temp. Use 1 ml gel per mg of the Cys-containing peptide or 1-5 mg protein per ml of the Cys-link Agarose. A wide bore pipette (10 ml) or 1 ml pipet tips cut at the bottom can be used to take agarose or simply poured off the column. It is possible to do the coupling protocol directly in the column. Store the remaining gel in a separate brown tube and store at 4oC.
2. Drain the storage buffer and add 4-gel bed volume (for 5 ml Agarose, add 20 ml of the coupling buffer and let it drain. Do not allow the gel to dry. Use the supplied bottom cap to stop the flow.
3. **Peptide coupling**-Dissolve peptide in coupling buffer (prepare 1-5 mg/ml solution). If desired, small sample can be saved for A280 or protein measurement before coupling.
4. Completely drain the Cys-link column and replace the bottom cap and add the peptide solution to the column. Replace the top-cap and seal it tightly using parafilm. This step can also be performed in 15-ml tube.
5. Mix the gel with the peptide solution using end-to-end mixing for 15-20 min at room temp. Allow the tube or the column to stand upright for an additional 30 minutes at room temp without mixing.