Most 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. 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 also affect the antibody activity. Suitability of a given protocol must be evaluated for a given protein.

Trouble Shooting and Common Problems

1. 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 should be tested.

2. It is very important to monitor the antibody titer or protein concentration at every step of purification. For example, if most of the antibody or protein is recovered in the unbound fraction then this would indicate a loss or inactivation of the bound antigen, or no binding of the antigen to the column. If antibody is bound to the column and the unbound fraction does not contain significant amount but there is still no recovery in the eluted fraction then a different elution protocol should be tried.

3. Often eluted proteins or antibodies may become inactive during the purification. A different elution protocol(high pH or Urea etc) should be tested.

4. 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 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.

Related items available from ADI

#110100-CG	Preactivated Cys-Link-Affinity Gel Sepharose
#110200-NG	Preactivated N-Link-Affinity Gel Agarose
#110300-AF	Affinity purification buffer kit for 10-20 antibody purifications
80220	Horseradish peroxidase (HRP) conjugation kit for antigen/antibodies
80230	Antibody/Protein conjugation to FITC (sufficient for 2 reactions)
80240	Peptide/Protein conjugation to Pre-activated BSA Kit (for 2 reactions)
80250	Peptide/Protein conjugation to Pre-activated KLH Kit (for 2 reactions)
80300	Protein (antigen/antibodies) Biotinylation Kit
80160	ELISA Kit for the detection of Rabbit Antibodies in serum, 75 min

Instruction Manual 110300-AF

Affinity purification buffers Kit

Cat. # 110300-AF

For most antigen-antibody based affinity purification using Agarose-based matrices

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Affinity purification buffer kit # 110300-AF

(Suitable for ADI's N-link #110200-NG for coupling proteins/peptide with via free amino group and Cys-link #110100-CG gel for coupling via the free cysteine groups)

Kit Contents

1. Binding buffer # 110300-1 (10X) , 50 ml
2. Elution buffer # 110300-2 (10x), 25 ml
3. Neutralization buffer #110300-3 (25 ml)

Sufficient reagents are provided for ~10 purification using ~5-6 ml affinity gel.
Form and storage: Upon receipt store at 4oC for 6 months.

Additional material required but not supplied

- Deionized or distilled water, Pipettes Tubes, beakers
- Wash buffer (prepared by diluting binding buffer # 1 1:100 (5 ml stock in 495 ml water and add 15 g NaCl. Mix to dissolve. Prepare as needed.
- Dialyses tubes, PBS, pH 7.4

Affinity purification time : 2-4 hrs at room temp.

Introduction

Antibodies to proteins are often made using short synthetic peptides (~20-25 amino acids) or purified or recombinant proteins containing fusion-tags (GST, MBP, His etc). The antigenic peptides must be covalently coupled to the carrier proteins such as KLH or BSA using the free amino, carboxyl or free cysteine. Antibodies are produced to the carrier proteins or the fusion tags along with the injected proteins or peptides. 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. the antigen (peptide or protein)-linked agarose gels are prepared to remove the unwanted antibodies (carrier protein or fusion tags or host animal proteins). Affinity purified antibodies usually work better in most immunoassays (ELISA, Western, IHC/IF, IP etc). Purified antibodies can also be linked to Agarose gels to enrich or purify target.

In general, affinity gel is allowed to bind the antigen or antibody. A washing step is performed to remove the unbound proteins. An elution buffer is used to disrupt the antigen or antibodies from the affinity gels. Typically, low or high pH buffers or Chaotropic agents (GdnHCl or Urea, SDS) can be utilized to disrupt the antigen-antibody bound to the affinity gel. There is no single method that will be applicable to all situations due to sensitivity of the protein to temperature, pH, salts or detergents. Therefore, protein binding, washing, and elution must be designed for a given protein or antibody purification.

This affinity purification kit is based upon most common method of purifying antibodies or proteins using low pH elution. Purified proteins or antibodies should be tested in a functional assay (ELISA, Western, IHC/IF etc) to determine the efficacy of the affinity purification.

A general protocol for the purification of antibodies using antigen-Agarose gels

Note: The same protocol can be utilized for purifying antigen using antibody-agarose affinity gels. Purification of antigen requires more attention paid to sensitivity of a given protein to temperature, salts, pH etc. Typically, affinity matrices (1-10 ml) is added to an appropriate affinity column. All steps are performed at room temperature but they can be done at 4oC if desired.

1. **Antibody Binding**-Antiserum should be diluted. 1:1 with 1x binding buffer and filtered before applying it on to the gel. It is a good idea to reapply the unbound solution several time to allow more binding (10-50 ml antiserum can be used per run for gels that contain ~1-5 mg peptide/proteins coupled to 1-5 ml gel). Collect the unbound fraction to analyzed protein or antibody titer by ELISA. Allow the column to drain completely (do not let it dry).

2. **Wash** column with 10 bed-volumes (if affinity gel is 5 ml then 1-bed volume=5 ml) of **Wash buffer** (Buffer # 1 + 0.5M NaCl, prepare as needed). At the end of the wash, collect 1 ml in a clean tube to determine A280 (should be <0.1 or background levels).

3. **Elute** bound proteins in 1X elution buffer (Add at least 3-bed volumes) and collect fractions (0.5-2 ml or more depending upon the column size). Normally, elution is done in tubes that containing 1/10th volume of 1X neutralization buffer (for 1 ml fraction add 100 ul of 1x buffer) to immediately neutralize the proteins.

4. The column is regenerated in 1x binding buffer by passing 5-10 volume. Close the bottom cap and add 5 ml of buffer containing 0.1% azide or other preservatives. Typically, affinity columns can be re-used several times (10-20 times depending upon the nature or the bound peptide or protein). Store affinity column at 4oC (never freeze the matrix).

5. **Purified antibody is monitored** in fractions by A280. Pool all fractions containing high protein. It is a good idea to add 0.1% protease-free BSA to stabilize antibodies. Do not add BSA if antibodies are to be coupled to HRP, FITC or agarose. Eluted antibodies can be dialyzed against appropriate buffer (PBS, ph 7.4 or other buffer, overnight at 4oC) and protein concentration determined. If necessary, antibody may be concentrated.

6. Antibody titer should be tested by ELISA or other technique in the starting solution, unbound, and eluted antibody to give us idea about binding capacity of the column and how much is remaining in the unbound fractions. Antibody activity may be lost during affinity purification while one may still show lots of protein.