AFFINITY ISOLATION SYSTEM
RAT IgG
K4091R-10ml (New)

(The review of a publication such as Affinity Chromatography: Principles and Methods, Pharmacia Fine Chemicals, Ljungforetagen AB, Orebro June 1979-1 is recommended before attempting antibody purification.)

Monoclonal antibodies may be isolated from either ascites fluid or tissue culture supernatant by utilizing affinity chromatography techniques.

Optimally, the solution containing the antibodies to be purified should be in a volume about twice the bed volume of the affinity column. Therefore, if 5 ml of ascites fluid is to be used, dilute this ascites fluid to 20 ml with PBS. If tissue culture supernatants are used, the volume should be concentrated down, if possible.

The following guide is a simple procedure developed and utilized by American Qualex. By using this procedure, the affinity isolation of specific antibody should be relatively easy. However, because of the highly variable nature of antibodies to chaotropic disruption, **AMERICAN QUALEX CANNOT GUARANTEE THE YIELDS YOU WILL OBTAIN WITH YOUR ANTIBODY PREPARATION.**

Enclosed will be found the following:

1. 10 ml bottle of Anti-Rat IgG Agarose Beads
2. 25 ml 20X PBS Concentrated Buffer Solution (To make 1 liter of phosphate buffered saline solution)
3. 50 ml of 10X Eluting Buffer (1.0 M Glycine pH 2.1)
4. 1.0 ml Rat Blocking Serum

Additional Materials Required:

1. 50-100 ml sintered glass funnel with vacuum flask

American Qualex Antibodies

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TECHNICAL NOTES

It has been observed that with some monoclonal antibody subclasses, the affinity column will bind irreversibly due to certain populations of ultra-high affinity antibodies incorporated within the column. By washing the column with the provided Blocking Serum to block these antibodies, the column can be used for monoclonal antibody purification. Use the provided Blocking Serum in the protocol as if it were ascites fluid and proceed as outlined in the protocol.

It will not be necessary to keep the column eluate for the blocking serum, but wash the column thoroughly before adding ascites or supernatants.

Blocking has not been done in advance to preserve the activity of the column for the user.

The following notes will increase the probability of success of your isolation attempts:

1. After elution of the antibody with the eluting buffer, adjust the pH of the antibody solution to between 6.0 and 8.0 with solid Tris, Na2HPO4 or any similar basic buffering agent (use caution if NaOH is used; the pH cannot be readily controlled).

2. Affinity isolated antibodies aggregate readily; dialyze eluted antibody against a buffer solution containing 2X saline with pH between 8.0 and 8.5. High salts and basic pH will improve yield. Also, avoid freeze-thawing. Every time that the antibodies are thawed, there is some degree of aggregation.

PROTOCOL

1. Prepare Eluting Buffer solution by diluting 50 ml to 500 ml with distilled water.

2. Prepare washing buffer by diluting 25 ml of the 20X PBS concentrate up to 500 ml.

3. Prepare ascites fluid or tissue culture supernatant to a total volume that you will use (but our suggestion is to consider keeping between 20 ml to 40 ml), and place in a beaker.

4. Add the Agarose Beads to the antibody solution. Rinse the bottle with a few milliliters of saline (0.85% NaCl).

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PROTOCOL (CONTD.)

5. Gently stir for two hours. Stirring should be such that beads continually remain in solution.

6. Place the scintered glass funnel over a 1 liter vacuum flask and rinse funnel with approximately 50 ml of PBS. Discard rinse. Slowly pour contents of beaker into funnel allowing fluids to drain by gravity or vacuum. Caution: do not allow beads to dry completely!

7. Collect fluid from flask and check for antibody. DO NOT DISCARD until determining that no antibody is present in flask contents!

8. Wash beads with PBS solution (approximately 50 ml to 100 ml) until effluent directly from filter funnel is free of protein (less than 0.01 OD units at A280nm).

9. Allow all buffer to drain from funnel. Do Not Draw Off Buffer Being Held By Beads!

10. Slowly pour Eluting Buffer (approximately 25 ml to 50 ml) over beads while stirring. A gentle vacuum can be used here to expedite elution. Solid Tris or Na2HPO4 may be added to eluting flask to decrease time antibody is exposed to acid conditions.

11. Remove contents from flask and immediately dialyze against a high salt buffer (0.1 to 0.2M) with a pH between 8.0 and 8.5. Continue to wash the affinity column with PBS until the effluent is free of protein.

12. Remove contents of flask and immediately dialyze against a high salt buffer with a pH between 8.0 and 8.5.

13. Concentrate protein solution to desired level.

- FOR RESEARCH ONLY -
STORE AT 2 – 8°C

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COMPONENTS PROVIDED WITH KIT

KIT LOT #: N050E

43 -	N049E	- Anti Rat IgG Agarose Beads **Goat** **(Note: the goat species used for production for this antibody on these agarose beads is Capra aegagrus hircus (NOT WILD), a raised goat species that is NOT included in the Washington Convention.)**	10 ml
38 -	R282L	- PBS Buffer Concentrate (20X)	25 ml
54 -	N002A	- Eluting Buffer Concentrate (10X)	50 ml
55-	N048E	- Blocking Serum (Rat)	1.0 ml

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