

Purification of Mouse Mono-antibody from Ascites Fluid

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Solution:

- 1) 5 M NaCl, 0.05% Sodium Azide
- 2) Saturated sodium borate water solution
- 3) 3.0 M NaCl, 50 mM sodium borate, 0.05% Sodium Azide, pH 8.9
- 4) 3.0 M NaCl, 10 mM sodium borate, 0.05% Sodium Azide, pH 8.9
- 5) 1.0 M Tris Buffer, pH 8.0
- 6) 100 mM Glycine-HCl buffer, 0.05% Sodium Azide, pH 3.0

*It is recommended to filter all solutions here by passing them through a 0.22 µm filter before use

1. Thaw 1 ml mouse ascites fluid.
2. Centrifuge at 12,000 rpm (.....g) on mini-table centrifuge for 1 minute at RT.
3. Remove the supernatant to another tube, discard the pellet.
4. Add 2 ml 5 M NaCl (containing 0.05% Sodium Azide) and 300 µl saturated sodium borate water solution to the supernatant. Mix the tube gently, then check the pH (It should be higher than 8.0).
5. Pass the ascites fluid solution through a protein A bead column (Suggest using 1 ml column).
6. Repeat step 5 at least 3 times.
7. Wash the bead (column) with 10 column volumes of 3.0 M NaCl, 50 mM sodium borate, pH 8.9 (containing 0.05% Sodium Azide).
8. Wash the bead (column) with 10 column volumes of 3.0 M NaCl, 10 mM sodium borate, pH 8.9 (containing 0.05% Sodium Azide).
9. Elute the column with 100 mM Glycine-HCl buffer (pH 3.0). Add this buffer stepwise, approximately 500 µl per sample. Collect the elute in 1.5-ml conical tube containing 50 µl of 1 M Tris buffer (pH 8.0). Mix each tube gently to bring the pH back to neutral.
10. Identify the immunoglobulin-containing fraction by absorbance at 280 nm (1 OD = 0.8 mg/ml) or by Bradford dye binding spot test.

References:

1. Ey et al.1978, Isolation of pure IgG₁, IgG_{2a} and IgG_{2b} immunoglobulins from mouse serum using protein A-Sepharose. *Immunochemistry*, Vol 15, pp 429-436.
2. Harlow, E., and D. Lane. 1988. *Antibodies: a laboratory manual*, pp 309-311. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Notes:

How to use Hitrap rProtein A FF 1ml affinity column (Amersham Biosciences)?

- 1) The column is prepacked with rProtein A Sepharose Fast Flow for purification and fraction of IgG subclasses and IgG from different sources. The column can not be opened or refilled. Designed for use with a syringe, peristaltic pump, FPLC System, etc.
- 2) The sample should be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column. Never apply a turbid solution to the column.
- 3) It is recommended to use a flow rate of 1 ml/min for Hitrap rProtein A FF 1 ml column.

- 4) Fill the syringe or pump tubing with start buffer. Remove the stopper and connect the column to the syringe (with the provided adaptor), or pump tubing, "drop to drop" to avoid introducing air into the column.
- 5) Remove the twist-off end.
- 6) Wash out the ethanol preservative with at least 5 column volumes of distilled water or binding buffer.
- 7) Regenerate the column with 5 column volumes of elution buffer.
- 8) Equilibrate the column with 5-10 column volumes of binding buffer.
- 9) Apply the sample, using a syringe fitted to the luer adaptor or by pumping it onto the column.
- 10) Wash with 5-10 column volumes of binding buffer or until no material appears in the effluent.
- 11) Elute with elution buffer.
- 12) The reuse of HiTrap rProtein A FF depends on the nature of the sample and should only be performed with identical monoclonals to prevent cross-contamination.
- 13) Before storage, it is recommended to wash the column with five column volumes of 20% ethanol to prevent microbial growth. Seal the column with the supplied stopper and domed nut. Store the HiTrap rProtein A FF column at +4 to 8 °C.