Antibody Affinity Purification

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[Abstract] Antibody purification is performed to concentrate and enrich antigen-specific antibodies and lower the background signal during detection by removing any non-specific proteins. Affinity purification makes use of specific binding interactions between molecules, and is very simple and efficient.

Materials and Reagents

1. PBS
2. NaCl
3. KCl
4. Na_2HPO_4
5. KH_2PO_4
6. Glycine
7. HCl
8. Tris-HCl
9. BSA
10. Azide
11. Affinity resin
12. Sepharose
13. 10x PBS (see Recipes)
14. 1x PBS + 1 M NaCl (see Recipes)
15. 0.1 M glycine-HCl (pH 2.8) (see Recipes)
16. 1 M Tris- HCl (pH 8.5) (see Recipes)

Equipment

1. Centrifuges
2. Affinity resin
3. Spectrometer

Procedure

1. Mix serum with affinity resin (protein coupled to sepharose). If using whole serum, dilute 1: 10 with PBS. Incubate overnight with gentle agitation.
2. Pour resin into column and collect the flow through. This is 1x pre-absorbed immune serum.
3. Wash the column with 1x PBS (pH 7.5) until the OD_{280} is zero.
4. Wash the column with PBS + 1 M NaCl to elute non-specifically absorbed material until the OD_{280} is zero.
5. Wash the column with PBS until the OD_{280} is zero.
6. Wash the column with 10 volume of 0.1 M glycine-HCl to elute specifically bound material. The acid eluant is neutralized by placing an aliquot (1/10 of fraction volume) of 1 M Tris-HCl (pH 8.5) into the fraction collection tubes. This is done prior to elution with acid. Collect the entire trailing peak-the highest affinity antibodies are released most slowly.
7. Wash the column with PBS until the OD_{280} is zero.
8. Pool the anitbody fractions and dialyze overnight against PBS/azide. After dialysis, check the OD, add BSA to 1%, aliquot and store at -70 °C.

Recipes

1. 10x PBS (pH 7.5)
   40 g NaCl
   1 g KCl
   7.2 g Na_2HPO_4
   1.2 g KH_2PO_4
   500 ml
2. 1x PBS + 1 M NaCl
   25 ml 10x PBS
   14.61 g NaCl
   250 ml
3. 0.1 M glycine-HCl (pH 2.8)
   0.75 g glycine in 100 ml, pH with 1 N HCl, make fresh.
4. 1 M Tris- HCl (pH 8.5)
   2.4 g Tris in 20 ml

References

1. Puig, O., Caspary, F., Rigaut, G., Rutz, B., Bouveret, E., Bragado-Nilsson, E., Wilm, M. and Seraphin, B. (2001). The tandem affinity purification (TAP) method: a general procedure of protein complex purification. *Methods* 24(3): 218-229.

