Antibody Purification (small scale)

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Abstract

This protocol described a easy procedure to purify anti-sera in small scale batch. Protein A or protein G beads can be used depending on the immunoglobulin types.

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Protocol

Step 1.
Centrifuge anti-sera @ 4ºC,14,000g,10min.

NOTES
Vivian Liu 20 Aug 2015
This step is to remove particulates in the serum.

ANNOTATIONS
Doug Holbrook 04 Sep 2015
Some more information

Step 2.
(Optional) Filter through 0.2um filter.

NOTES
Vivian Liu 20 Aug 2015
This step is to remove lipids in the serum.

Step 3.
Apply protein A or protein G beads gently to Bio-Rad empty gravity column.

NOTES
Vivian Liu 20 Aug 2015
anti-sera:beads slurry=1:1(v/v)

Step 4.
Set up the column on a vacuum manifold. Equilibrate column with 10 volumes of ice cold Binding Buffer.

NOTES
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Use vacuum to pack beads tightly.
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Binding Buffer: 1xPBS

Step 5.
Dilute anti-sera with 2 volumes of Binding Buffer.

Step 6.
Let diluted anti-sera go through the column by gravity.

NOTES
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No vacuum here.

Step 7.
Collect flow through and repeat step 6 once.

Step 8.
Wash column with 10 bed volumes of ice-cold Binding Buffer.

Step 9.
Elute antibody with 5 bed volumes of room temperature Elution Buffer.

NOTES
Vivian Liu 21 Aug 2015
Elution Buffer:
100mM citric acid,PH3.0 for protein A beads;
100mM Glycine-HCL,PH2.7 for protein G beads.

Step 10.
Fill up each collecting tube with 200 µl 1M Tris-HCl (PH9.0).

Step 11.
Collect 1ml of flow through in each collecting tube. Save for antibody activity test.

NOTES
Vivian Liu 21 Aug 2015
The PH9.0 Tris-HCl in the collecting tube will take the PH of flow through to around 7.

Step 12.
Re-equilibrate column with 10 bed volumes of Binding Buffer. Store Column at refrigerator for later use.