Purification of ICAM-1

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Purpose

The purification of ICAM-1 on R6.5 anti-ICAM-1 column.

Materials

- Triethylamine
- NaCl
- Octyl Glucoside (n-octyl-β-D-glucopyranoside Sigma Cat. No. O-8001)
- Tris-Base
- NaN3
- Tris-HCl
- PMSF (PhenylMethylSulfonylFluoride)
- Aprotinin
- Triton-X 100
- 100% EtOH
- Human IgG Column
- R6.5 mAb Column
- 5ml Falcon 2054 tubes

Pre-wash Buffer / Elution Buffer (TEA-saline pH 11.0 / 1% Octyl Glucoside)

- Triethylamine 3.5ml (Pipette in fume hood using glass pipette)
- NaCl 4.4g
- H_2O 450ml

Adjust pH to 11.0 with concentrated HCl. **Be careful!!! Adding concentrated acid to strong alkaline may cause a violent reaction.** Add dH_2O to 500ml final volume.

Note: Since octyl Glucoside is very expensive, take 100ml of this buffer and add to it 1g of Octyl Glucoside. This will be more than sufficient for one run

Equilibrium Buffer (Tris-saline pH 7.5 @RT {pH 8.0 @ 4ºC})

- Tris-Base 0.76g
- NaCl 4.4g
- dH_2O 450ml

Adjust pH to 7.5 with HCl. Add dH_2O to 500ml final volume. Add 0.03% NaN3.

Wash Buffer (Tris-saline pH8.0 / 1% Octyl Glucoside)

- Tris-HCl 0.26g
- Tris-Base 0.41g
- NaCl 4.4g
- dH_2O 450ml

Adjust pH to 8.0 using HCL. Add water to 500ml final volume.

Add 1g Octyl Glucoside to 100ml of Wash Buffer.

Add 1ml freshly prepared 100mM PMSF to each 100ml Wash Buffer. Final concentration of 1mM.

Add 1 ml 100X Aprotinin (20 TIU/ml) for each 100ml Wash Buffer. Final concentration of .2 TIU/ml.

Note: PMSF must be prepared fresh each time. Add 0.87g of PMSF to 50ml of 100% EtOH. **Be careful !!! PMSF is highly toxic.**

Elution Neutralization Buffer (1M Tris-HCl pH6.8)

- Tris-Base 12.1g
- dH_2O 80ml

Adjust pH to 6.8 with concentrated HCl. Add dH_2O to final volume of 100ml.

Stripping Buffer (TEA-saline pH 12.0 / 0.1 % Triton-X 100)

- Triethylamine 3.5ml (Pipette in fume hood using glass pipette)
- NaCl 4.4g
- H_2O 450ml

Adjust pH to 12.0 with concentrated NaOH. Add dH_2O to 500ml final volume.

Add 0.5ml Triton-X 100. Mix well.

Procedure

1. Pre-wash column with 5 column bed volumes of Pre-wash Buffer to pre-elute any materials that might leach from column. Minimize length of time column is exposed to Pre-wash Buffer because of the harsh denaturing conditions at pH 11.0.
2. Equilibrate the columns with 5 column bed volumes of Equilibration Buffer to return the column to pH that supernatant will be loaded at.
3. Assemble the column as by attaching Human IgG column to R6.5 column.
4. Load supernatant overnight at flow rate not higher than 1ml/minute. The flow rate can be slower, and is desirable for maximum binding.
   Note: It is a good idea to include a "safety loop" to prevent a the column from drying out it runs out of liquid.
5. Separate the two columns.
6. Wash R6.5 column with at least 10 column bed volumes of Wash Buffer. At same time strip the Human IgG column with 5 column bed volumes of Stripping Buffer.
7. Equilibrate Human IgG column with 10 column bed volumes of Equilibration Buffer and store at 4ºC.
8. Before beginning elution step of R6.5 column, set up the collection tubes. Use the 5ml Falcon 2054 tubes. On each tube number and mark off the 3ml mark. Add 0.3 ml of the Elution Neutralization Buffer.
9. Elute the R6.5 column 50ml of Elution Buffer. Elute 3ml in each prepared collection tube. Mix each tube immediately after elution and cap with parafilm to prevent evaporation during storage.
10. Strip the column with 5 column bed volumes of Stripping Buffer.
11. Equilibrate the column with 10 column bed volumes of Equilibration Buffer and store at 4ºC
12. O.D. fractions to determine which one have highest concentration of protein
13. Run protein gel to check concentration and to make sure you have the protein you want.