Antibody Purification from anti-Ntl Serum

Reagents and Supplies

2x YT broth + glucose & ampicillin
Per liter:
16 g Tryptone
10 g Yeast Extract
5 g NaCl
2 g Glucose
100 mg Ampicillin
(Autoclave)

0.1M IPTG stock
238 mg IPTG
10 mL Water
(Filter sterilize and store at 4°c)

Column Buffer
20 ml 1.0 M Tris-HCl, pH 7.4
11.7 g NaCl
2.0 ml 0.5 M EDTA
0.7 ml β-mercaptoethanol
975 mL Water

BL21 competent cells
LB+Amp agar plates
LB broth
Triton X-100
Ethanol
Amylose Resin (New England Biolabs)
Maltose
Amicon Concentrator (Millipore)
Slide-A-Lyzer Dialysis Cassette (Thermo Scientific)
1x PBS
AminoLink® Immobilization Kit (Thermo Scientific)
0.1 M Glycine-HCl, pH 3
1 M Tris, pH 9

Procedure

Transformation

1) Thaw BL21 competent cells on ice.
2) Add 2 µL ntl plasmid vector to a microcentrifuge tube containing ~100 µL cells.

3) Heat shock the cells at 42°C for 40 seconds.
4) Add 1 mL LB then transfer to a larger tube.
5) Rescue at 37°C shaking for 1 hour.
6) Spread 20 µL, 100 µL, and 200 µL on LB+Amp plates at 37°C ON.

Antigen Expression & Affinity Chromatography

1) Grow up an 80 ml overnight culture of cells transformed with the ntl plasmid in LB broth.
2) Inoculate 1 liter of 2x YT broth + glucose & ampicillin with the overnight culture and grow up for 2 hour at 37°C then 1 hour at 30°C (Check for OD_{600} 0.8-1.0).
3) Add IPTG to a final concentration of 0.5 mM (5 mL of a 0.1 M stock in H_2O), and continue to grow at 30°C for 6 hours.
4) Harvest the cells by centrifugation at 4000 x g for 20 minutes and discard the supernatant.
5) Resuspend the cells in 50 mL of Column Buffer and pipette to completely suspend the pellet.
6) Freeze sample in a dry ice-ethanol bath (or overnight at -20°C), then thaw in cold water.
7) Sonicate in short pulses 8 x 15 seconds in an ice-water bath.
8) Add 20% Triton X-100 to a final concentration of 1% and mix gently at 4°C for 30 min to aid in the solubilization of the fusion protein.
9) Centrifuge at 12,000 x g for 30 minutes, 4°C.
10) Pass the supernatant (gravity flow) over a 5 mL amylose resin column packed with 8 column volumes of Column Buffer.
11) Wash the column with 12 column volumes of Column Buffer.
12) Elute the column with Column Buffer + 20mM maltose, collecting 15 fractions of 1 mL each. Fusion protein is eluted by competition with the free maltose.
13) Detect fractions containing protein by A_{280} and SDS-PAGE gel analysis; the *ntl* fusion protein is 89.2 kD in size.
14) Pool protein-containing fractions and concentrate to about 1 mg/mL in an Amicon Centricon or Centriprep concentrator.

Antigen Immobilization & Affinity Purification of Antibody

1) Load the concentrated protein into a Slide-A-Lyzer dialysis cassette and perform an 18 hour dialysis in 1 L of PBS, changing the PBS twice, to thoroughly remove Tris from the protein solution. Any residual primary amines will compete with the intended protein-coupling reaction.

2) Follow “Procedure for Immobilizing a Protein to AminoLink Column” for the AminoLink® Immobilization Kit. At least 2 mg of fusion protein should be used per mL of AminoLink Column resin.
3) Follow “General Protocol for Affinity Purification of Protein” from Thermo Scientific. Up to 1 mL of *ntl* serum can be purified per mL of AminoLink resin bed-volume in the prepared column. The column may be regenerated to purify more serum. Do not overload the column.
4) Elute with 0.1 M Glycine-HCl in 200 μL fractions. The antibody should elute over the first 3 mL.
5) Neutralize collected fractions with 10 μL of 1 M Tris; pH 9.
6) Analyze by A_{280} to find protein containing fractions and test these fractions for purity by immunostaining and western blot.