rAB Expression

1.0 Introduction

This method is intended for the small (50-100ml) expression and purification of rABs that have been cloned into the pBL166 rAB expression vector.

For the most up to date version of this protocol please visit: http://recombinant-antibodies.org/protocols/pbL166

2.0 Materials

Glassware/Plasticware

250 mL baffled flasks

50 mL Conical Tube (Bioexpress, LLC C-3394-4)

Econo-pac 1.5x12cm Chromatography Column (Biorad 732-1010)

15ml Conical Tube (Bioexpress, LLC C-3394-1)

14ml Disposable culture tube (VWR 60818-703)

Reagents

Name	Company	Catalog Number
Terrific Broth	Fisher Scientific	50489053
Glycerol	Sigma-Aldrich	G7893-4L
Carbenicillin	Gold Biotech	C-103-100
Chloramphenicol	Teknova	C0325
C43(DE3) *E. coli*	Lucigen	60446-1
LB agar plates with 50ug/ml Carbenicillin		
Isopropyl-beta-D-thiogalactoside (IPTG)	Gold Biotech	12481C100
B-per lysis buffer	Pierce	78266
DNaseI 2500U/ml	Pierce	900830
PBS	VWR	16777-251
rProtein A Sepharose	Ge Healthcare	17-5138-01

Solutions

1X TB Expression Media

1X Sterile Terrific Broth made according to manufacturers instructions and add Glycerol (0.4% Final), Carbenicillin (50ug/ml Final), Chloramphenicol (50ug/ml Final)

Elution buffer

100mM Acetic Acid

Neutralization Buffer

1M Tris pH 11

3.0 Transformation

3.1 Transform <50ng sequence verified expression plasmid into C43 (DE3) chemically competent cells using standard laboratory transformation protocols and plate on LB Carb

4.0 Expression

4.1 Pick an isolated colony from step 3.2 above into 5ml LB+50ug/ml Carb and Chlor into 14ml culture tube and grow 16-18 hours in shaking incubator at 37C

4.2 Dispense 100ml 1X TB expression media into 250ml baffled flasks

4.3 Inoculate with 200ul overnight culture from step 4.1

4.4 Incubate culture at 37C in shaking incubator (200-250 rpm) to an OD_{600} of 0.6-0.8

4.5 Add IPTG to final concentrations of 1mM then reduce incubator temperature to 25C, and incubate for an additional 16-18 hours

4.6 Transfer ½ the volume of culture to a 50 mL Conical tube and pellet cells at >4000xg for 20 min.

4.7 Discard supernatant and repeat to obtain a single pellet

4.8 Discard remaining supernatant. At this point the pellet can be frozen for purification at a later date of lysed for purification as per the following step

5.0 Purification

5.1 Resuspend and lyse pellet by the addition of 5 mL of B-per lysis buffer and add 5ul DNaseI

5.2 Incubate at room temperature for 10 minutes

5.3 Add 5ml PBS to lysed cells

5.4 Transfer lysed cells with tightly closed tube cap to 60C water bath and incubate for 20 minutes

5.5 Centrifuge at 30,000xg for 20 minutes to pellet cell debris

5.6 Meanwhile, prepare Protein A purification column by adding 1ml Protein A sepharose slurry to a 15ml GF column. Wash Protein A with 10ml ddH2O then equilibrate with 15ml PBS

5.7 Transfer clarified cell lysate to the equilibrated and capped Protein A column and allow binding to proceed for 30 min at $4^{o}C$ with occasional mixing of the resin with the supernatant

5.8 Drain the supernatant from the column retaining the flow-through and wash the resin bed with 40 ml 1X PBS

5.9 Add 1ml of 1M Tris pH 8 added to 15ml Falcon tube for collecting eluted protein

5.10 10ml Fab elution buffer (above) added to each column

5.11 Concentrate and buffer exchange by dialysis or spin concentration into PBS

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