Biotinylated rAB Expression

1.0 Introduction

This method is for the mid-scale expression and purification of site-specifically biotinylated Fabs from the RH2.2-Avi pTAC-based IPTG-inducible, Avi-tagged vector.

For the most up to date version of this protocol please visit: http://recombinant-antibodies.org/protocols/rh22-avi

2.0 Materials

Glassware/Plasticware

250 mL baffled flasks (Fisher Scientific, 10-140-6A)
50 mL Conical Tube (Bioexpress, LLC C-3394-4)
15 mL Biorad protein purification columns (EconoPac, 732-1010)
1.7 mL Eppendorf tubes (Axygen, MCT-150-C)

Reagents

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sodium chloride  
phosphoric acid  
lysozyme  
benzonase  
Triton X-100  
protease inhibitor cocktail tablets  
Protein A sepharose  
IPTG  

BirA-transformed, chemically competent T1\textsuperscript{r} BL21 E. coli

Solutions

1X KCM
KCl (500 mM)  
CaCl\textsubscript{2} (150 mM)  
MgCl\textsubscript{2} (250 mM)  

2YT media
1% yeast extract (10 g)  
1.6% bio-tryptone (16 g)  
86 mM NaCl (5 g)  

Make up volume to 1L and adjust pH to 7.0

20X salt stock
500 mM Na\textsubscript{2}HPO\textsubscript{4} (70.98 g)  
500 mM KH\textsubscript{2}PO\textsubscript{4} (68.05 g)  
1M NH\textsubscript{4}Cl (53.4 g)  
100 mM Na\textsubscript{2}SO\textsubscript{4} (14.20 g)  

Mix with heating and sterile filter or autoclave until dissolved and make up to 1L. Store at RT

NZY media
1% N-Z-Amine (10g)  
0.5% yeast extract (5 g)  

Mix in 500 mL and stir until dissolved

Add 50 mL of 20X salt  
Add 2 mL of 1M MgSO\textsubscript{4}  

Make up volume to 980 mL and autoclave

Lysis buffer:
50mM Tris  
150mM NaCl  
1% Triton X-100  
1mg/ml lysozyme
2mM MgCl2
10U benzonase

Elution buffer:
50mM NaH$_2$PO$_4$
140mM NaCl
100mM H$_3$PO$_4$
pH 2.5

3.0 Transformation

3.1 Thaw 25 μL of chemically competent BL21-BirA T1’ cells on ice
3.2 Add 50 ng of sequence-verified pTAC-based RH2.2_Avi-expression plasmid to a mixture of 4 μL of 5X KCM in 16 μL of MQ H$_2$O on ice
3.3 Chill mixture on ice for 10 minutes
3.4 Add 20 mL of chemically competent BL21-BirA T1’ cells to the mixture of KCM and DNA
3.5 Incubate 20 min on ice, transfer to the benchtop, incubate 10 min at RT, return to ice and incubate 2 min on ice

4.0 Expression

4.1 Following the last stage of transformation on ice, transfer the entirety of the cell/DNA mixture in to 25 mL of 2YT supplemented with 50 μg/mL carb and 5 μg/mL cmp in a 50 mL Falcon tube, leaving the lid loose but taped secure to allow gas transfer
4.2 Inoculate 1/40th volume (2.5 mL) of overnight culture into NZY media supplemented with 50 μg/mL carb + 5 μg/mL cmp + 25 μM biotin (100 μL) and 1/20th volume of 20X salt mixture
4.3 Grow 2-3 hr at 37°C, 200rpm until OD0.8-1.0 achieved
4.4 Induce culture with 1/1000th volume of 1M IPTG (i.e. 100 μL in to 100 mL of culture, final [IPTG] = 1mM )
4.5 Continue growing for 6-8 hrs at 30°C and shaking at 200 rpm
4.6 Transfer ½ the volume of culture to a 50 mL Falcon tube and pellet cells at 8000 rpms 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 Lyse pellet by the addition of 10 mL of lysis buffer and nutation for 1.5-4 hrs at 4°C
5.2 To ensure complete lysis, mixture can be sonicated for 1 min at 40% intensity using a 1 min program of 5 seconds on and 5 seconds off.
5.3 Centrifuge the pellet lysate at 9000rpm for 20min.
5.4 Add 250 μl of a protein A sepharose slurry added to a 15 ml GF column.
5.5 Equilibrate resin with 25 mL of 1X PBS, drain and cap.
5.6 Transfer the lysate supernatant to the equilibrated protein A column and allow binding to proceed for 30 min at 4°C with occasional mixing of the resin with the supernatant.
5.7 Drain the supernatant from the column retaining the flow-through and wash the resin bed with 40 ml of 1X PBS.
5.8 Add 60 μl of 1M Tris pH 8 added to Eppendorf collection tubes to neutralize the elution buffer.
4.8 Add 300 μl Fab elution buffer (above) to each column and incubate 5 min before draining into 1.7 mL Eppendorf containing neutralization buffer.

5.9 Elution step can be repeated for a total elution volume of 600 μl neutralized with 1200 μl Tris.

5.10 Additional polishing steps can be carried out as necessary.

5.11 Columns can be regenerated with 10mL of 100mM H₃PO₄, washed with 20ml TBS, stored in 2ml 20% EtOH at 4°C.

Please send corrections, modifications and suggestions to shane.miersch@utoronto.ca