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

Name	Company	Catalog Number
N-Z Amine	Fluka	C02626-1KG
yeast extract	Bioshop	YEX401.1
bio-tryptone	Bioshop	TRP402.1
glucose	Sigma	G8270-1KG
carbenicillin (carb)	Bioshop	CAR544.10
chloramphenicol (cmp)	Bioshop	CLR201.100
biotin	Sigma	B4501-5G
Tween 20	Bioshop	TWN510.500
potassium chloride	Bioshop	POC308.1
calcium chloride	Bioshop	CCL302.500
magnesium chloride	Bioshop	MAG510.500
monobasic potassium phosphate	Bioshop	PPM302.1
dibasic sodium phosphate	Anachemia	84486-440
ammonium chloride	Amresco	0621-1KG
ammonium sulphate	Bioshop	AMP302
sodium sulphate	Bioshop	SOS513.500
magnesium sulphate	BDH	BDH0246-500G

sodium chloride	Bioshop	Sod001.10
phosphoric acid	Acros Organics	201140010
lysozyme	Bioshop	LYS702.25
benzonase	Novagen	71205
Triton X-100	Bioshop	TRX506.500
protease inhibitor cocktail tablets	Roche	11 836 170 001
Protein A sepharose	GE Healthsciences	17-1279-03
IPTG	Bioshop	IPT001.50

BirA-transformed, chemically competent T1^{r} BL21 *E. coli*

Solutions

1X KCM

KCl (500 mM)
$CaCL_2$ (150 mM)
$MgCl_2$ (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 Na2HPO4 (70.98 g)

500 mM KH2PO4 (68.05 g)

1M NH4Cl (53.4 g)

100 mM Na2SO4 (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 MgSO4

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_2PO_4

140mM NaCl

100mM H_3PO_4

pH 2.5

3.0 Transformation

3.1 Thaw 25 µL of chemically competent BL21-BirA $T1^r$ 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_2O on ice

3.3 Chill mixture on ice for 10 minutes

3.4 Add 20 mL of chemically competent BL21-BirA $T1^r$ 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 $1/40^{th}$ 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 $1/20^{th}$ volume of 20X salt mixture

4.3 Grow 2-3 hr at 37oC, 200rpm until OD0.8-1.0 achieved

4.4 Induce culture with $1/1000^{th}$ 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^oC 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.

4.6 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^oC

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.

5.2 Centrifuge the pellet lysate at 9000rpm for 20min.

5.3 Add 250 µl of a protein A sepharose slurry added to a 15 ml GF column.

5.4 Equilibrate resin with 25 mL of 1X PBS, drain and cap.

5.5 Transfer the lysate supernatant to the equilibrated protein A column and allow binding to proceed for 30 min at 4^oC with occasional mixing of the resin with the supernatant.

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

5.7 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 in to 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 H_3PO_4, washed with 20ml TBS, stored in 2ml 20% EtOH at 4^{o}C.

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

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