

Introduction

This protocol is “optimized” for 55244 cells and non his-tagged rAB on FPLC system from pFab007 expression vector using Phosphate Limiting Media (CRAP).

For the most up to date version of this protocol please visit: <http://recombinant-antibodies.org/protocols/pfab007>

Expression

1. Transform expression cells (strain 55244) with plasmid, do not plate, recover for 1h in SOC or 2YT media. Use 100% of the transformation to inoculate culture of 200mL CRAP/amp100 and grow O/N at 30°C, 220 rpm. Always start from fresh transformation; do not use frozen cell stock.
2. Heat the whole culture to lyse the cells. Add 0.1% Triton X-100 mix well and put the entire flask in the heated bath 60C for 30min and chill on ice for 5min.
3. Spin down 20000g, 1h, 4°C. Collect the supernatant and proceed to protein-A affinity purification by loading the supernatant directly on the pre-equilibrated protein-A affinity column. Supernatant should be filtered before loading to protect the column.

Protein A Purification

1. Regenerate 5ml rProtein A FF column (or 5ml Protein A HP) with 3 column volumes (C.V.) of 1M acetic acid. For low endotoxin level prep use 5ml of MabSelect resin sanitized with 0.1M NaOH.
2. Equilibrate column with 10 C.V. of running buffer (50mM Tris/500mM NaCl pH 8.0).
3. Load supernatant onto column at ~5ml/min.
4. Wash with 10 C.V. running buffer at 5ml/min or until OD280 reaches baseline, avoid excessive washing (>100C.V.).
5. Elute protein with 0.1M acetic acid at 3ml/min collecting 2 mL fractions. Note: The rAB usually elutes after the second 2 mL fraction of actual elution.
6. The eluted solution can be directly loaded onto SP-Sepharose HP without neutralization and/or desalting step. Note: The rAB is typically > 95% pure following protein A purification, and for most applications, it is sufficient. Hence, the cation exchange chromatography can be skipped. It is recommended to perform ion exchange if rAB is going to be used in live cells or crystallography. Proceed to dialyzing and storing the rAB in the buffer of choice (e.g. PBS, 20/140, pH 7.4 or any neutral pH buffer).
7. Regenerate protein-A column with 1M acetic acid and equilibrate with 5C.V. Of running buffer before storage.

10X CRAP Phosphate Limiting Media

Prepare the 10x media in a beaker and pour the exact volume of the media into an empty sterile flask. Filter sterilize 10x media. You can use hot water to dissolve media components.

10x Stock	1L	2L	4L
(NH ₄) ₂ SO ₄	35.7g	71.4g	142.8g
NaCitrate-2H ₂ O	7.1g	14.2g	28.4g
KCl	10.7g	21.4g	42.8g
Yeast Extract	53.6g	107.2g	214.4g
Hy-Case SF Casein	53.6g	107.2g	214.4g
ddH ₂ O	Up to 1L	Up to 2L	Up to 4L

1X CRAP Media

Add the following to make 1L of 1X media from 10X stock (do not store the 1X solution long term).

	1L	2L	4L
10X CRAP-Pi	100mL	200mL	400mL
1M MOPS pH 7.3	110mL	220mL	440mL
50% glucose	11mL	22mL	44mL
1M MgSO ₄	7mL	14mL	28mL
water	Up to 1L	Up to 2L	Up to 4L

Additional Information

You can obtain the 55244 strain from ATCC:<http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=55244&Template=bacteria>

55244 Genotype: tonA ptr3 deltaphoA deltaE15 delta(argF-lac)169 degP41 deltaompT; kanamycin-resistant; protease-deficient

Hy-Case SF: Sigma catalog number C9386.

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