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Production of GTPase Deficient RAB1A(Q70L) Protein

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We use this protocol and it's working

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Abstract

Bacterial expression of the GTPase deficient Q70L mutant of human RAB1A.

Attachments

844-2181.pdf
79KB

Materials

Materials and Reagents

- BL21 strain of *E. coli*
- pET vector containing 6x-His-RAB1A(Q70L)
- Ampicillin-resistant plates
- LB broth
- Isopropyl β-D-1-thiogalactopyranoside (IPTG)
- Phosphate Buffered Saline (PBS)
- Lysis buffer
- EDTA-free protease inhibitor tablet (Thermo Fisher)
- Phenylmethylsulfonyl fluoride (PMSF)
- Ni-NTA column
- Wash buffer (containing 300 mM imidazole)
- S75 10/300 column
- Liquid nitrogen

Lysis buffer

	A	B
	HEPES pH 7.5	25 mM
	NaCl	300 mM
	$MgCl_2$	2 mM
	TCEP	1 mM

Transformation and Colony Selection

1 Transform *E. coli* BL21 cells with the pET vector containing 6x-His-RAB1A(Q70L) by heat shock.

2 Plate the transformed cells on ampicillin-resistant plates.

3 Incubate the plates Overnight at 37 °C .

Pre-culture and Expansion

4 Inoculate a single colony into 10 mL of LB broth.

5 Incubate the culture Overnight at 37 °C .

Main Culture Preparation

6 Transfer the entire pre-culture into a 1 L culture flask.

7 Grow the 1 L culture at 37 °C with shaking at 220 rpm until the optical density (OD) reaches approximately 0.6.

Cooling and Induction

15m

8 Place the flask in an ice bath for 00:15:00 to cool rapidly. 15m

9 Induce protein expression by adding [M] 0.1 millimolar (mM) IPTG.

10 Continue incubating the culture Overnight (approximately 16 hours) at 18 °C with shaking at 180 rpm .

Cell Harvest and Pelleting

11 Harvest the cells by centrifugation at 4000 rpm in a BECKMAN Coulter centrifuge.

Cell Washing and Freezing

12 Wash the cell pellet once with PBS.

13 Pellet the cells again at 4000 x g .

14 Flash freeze the pellet in liquid nitrogen.

15 Store the frozen pellet at -80 °C until purification.

Cell Lysis

6m

16 Thaw the frozen cell pellet to Room temperature .

17 Resuspend the cells in lysis buffer.

Lysis buffer

	A	B
	HEPES pH 7.5	25 mM
	NaCl	300 mM
	$MgCl_2$	2 mM
	TCEP	1 mM

18 Add an EDTA-free protease inhibitor tablet (Thermo Fisher) and PMSF to a concentration of [M] 1 millimolar (mM) .

19 Lyse the cells by sonication using a 5-second on, 5-second off cycle at 50% power for a cumulative time of 00:06:00 . 6m

Clarification by Centrifugation 45m

20 Centrifuge the lysate at 17000 rpm, 00:45:00 to clarify. 45m

Protein Purification on Ni-NTA Column

21 Apply the supernatant to a Ni-NTA column (3x).

22 Wash the column until the wash buffer is free of protein, as measured by a Bradford assay.

23 Elute the protein using a wash buffer containing [M] 300 millimolar (mM) imidazole.

Size Exclusion Chromatography

24 Concentrate the eluted protein.

25 Apply the concentrated protein to an S75 10/300 column in buffer containing [M] 25 millimolar (mM) HEPES pH 7.5 , [M] 150 millimolar (mM) NaCl, [M] 1 millimolar (mM) $MgCl_2$, and [M] 1 millimolar (mM) TCEP.

Final Protein Concentration

26 Concentrate the purified protein to a final concentration of [M] 50 micromolar (µM) .

27 Divide the protein into 50 µL aliquots.

Cryopreservation

28 Flash freeze the protein aliquots in liquid nitrogen.

29 Store the frozen protein aliquots at -80 °C for future use.