Feb 06, 2025

Protocol for the production of RAB1A-10xHis in HEK293Gnti cells

DOI
https://dx.doi.org/10.17504/protocols.io.n2bvj9be5lk5/v1

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Protocol Citation: Annan Cook 2025. Protocol for the production of RAB1A-10xHis in HEK293Gnti cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj9be5lk5/v1

Protocol status: Working
We use this protocol and it's working

Created: February 05, 2025

Last Modified: February 06, 2025

Protocol Integer ID: 119639

Keywords: ASAPCRN, hek293gnti cells protocol, protocol for the production, cell

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000350

Abstract

Protocol for production of RAB1A-10xHis

Materials

Cell culture and transfection:
HEK 293T GNTi cells
pCAG RAB1A-10xHis DNA
Polyethyleneimine (PEI) (Polysciences) 20,000 Da
Hybridoma media (Thermo)
Freestyle Media
Buffers and chemicals:
Phosphate-buffered saline (PBS)
Lysis buffer:25 mM HEPES, pH 7.5, 200 mM NaCl, 2 mM $MgCl_2$,25 mM tris(2-carboxyethyl)phosphine (TCEP), 10% Glycerol
Protease inhibitor (EDTA-Free, Thermo Scientific)
Triton X-100
imidazole
Gel filtration buffer:25 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 1 mM TCEP
Equipment:
Bucket Centrifuge
Ultracentrifuge
Pyrex Dounce homogenizer
Rocker
NiNTA resin (2 mL)
S75 10/300 column
Liquid nitrogen

Cell growth and transfection

1 Grow 400 mL of HEK293T GNTi cells to a density of 2.0–2.2 × 10^6 cells/mL.

2 Transfect the cells with 400 µg of pCAG RAB1A-10xHis construct using a four fold molar excess of PEI in 80 mL Hybridoma

Cell harvesting

3 After 48 hours, pellet the cells by centrifugation at 2200 rpm.

4 Wash and gently resuspend the pellet with PBS, centrifuge again at 500 x g for 10 minutes, and flash-freeze the pellet in liquid nitrogen. Store at -80°C.

Purification

5 Thaw the cell pellet at room temperature.

6 Resuspend the pellet in lysis buffer (25 mM HEPES (pH 7.5), 200 mM NaCl, 2 mM $MgCl_2$, 1 mM TCEP, 10% Glycerol) and add an EDTA-free protease inhibitor tablet (Thermo Fisher).

7 Transfer the resuspended pellet to a Pyrex Dounce homogenizer and homogenize the cells with 30 strokes.

8 Add Triton X-100 to the homogenate for a final concentration of 1%.

9 Gently mix and rock the mixture at 4°C for 1 hour.

10 Centrifuge the lysate at 17,000 rpm for 45 minutes at 4°C.

11 Apply the supernatant to 2 mL of NiNTA resin, repeating the application 3 times.

12 Wash the resin until the wash is free of protein, as determined by a Bradford assay.

13 Elute the protein using wash buffer supplemented with 300 mM imidazole.

14 Concentrate the protein and apply it to an S75 10/300 column equilibrated with buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 1 mM TCEP).

15 Concentrate the protein to 50 µM.

16 Aliquot 50 µL of the purified protein and flash freeze in liquid nitrogen.