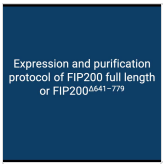

Jun 17, 2021

Expression and purification protocol of FIP200 full length or FIP200$^{\Delta 641-779}$

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1 Works for me Share dx.doi.org/10.17504/protocols.io.bvjjn4kn

ULK1 complex purification

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ABSTRACT

This protocol describes the expression and purification of FIP200 full length or FIP200$^{\Delta 641-779}$.

ATTACHMENTS

Expression and purification protocol of FIP200 full length or FIP200Δ641–779.pdf

DOI

dx.doi.org/10.17504/protocols.io.bvjjn4kn

PROTOCOL CITATION

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KEYWORDS

FIP200 full length or FIP200Δ641–779, Expression and purification protocol of FIP200 full length or FIP200Δ641–779, FIP200 full length , expression, purification, FIP200

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Jun 17, 2021

OWNERSHIP HISTORY

Jun 06, 2021 zahara93.zs

Jun 16, 2021 Xiaoshan Shi University of California, Berkeley

PROTOCOL INTEGER ID

50507

MATERIALS TEXT

Lysis Buffer: 50 mM Hepes pH=7.4; 1% Triton X-100, 200 mM NaCl, 1 mM $MgCl_2$, 10% Glycerol, 1 mM TCEP, EDTA-free Protease Inhibitors (Roche).
Wash Buffer 1: 50 mM Hepes pH=7.4, 500 mM NaCl, 1 mM $MgCl_2$,1 mM TCEP, 1% Triton X-100, 10% Glycerol;
Wash Buffer 2: 20 mM Hepes pH=8, 200 mM NaCl, 1 mM $MgCl_2$,1 mM TCEP;
Elution Buffer 1: 50 mM Hepes pH=7.4, 500 mM NaCl, 1 mM $MgCl_2$, 1 mM TCEP, 1% Triton X-100, 10% Glycerol, 50 mM Glutathione;
Elution Buffer 2: 20 mM Hepes pH=8, 200 mM NaCl, 1 mM $MgCl_2$, 1 mM TCEP, 50 mM Maltose;
Resin:

- Glutathione Sepharose 4B (GE Healthcare)
- Amylose resin (New England Biolabs).

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

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BEFORE STARTING

General information: expression system: human embryonic kidney (HEK) 293 GnTI suspension cells, medium: freestyle media + Anti-Anti; plasmid origin: Addgene 171410 or 17141; backbone: pCAG; resistance: Amp; insert: *Homo sapiens* FIP200 (synthetic gene); tags & cleavage sites: GST-TEVcs-FIP200-MBP, Ext coeff: 183470 $M^{-1}cm^{-1}$, MW 252 kDa

1- Protein expression: 2d 11h 10m

1 Transfect DNA in cells at a concentration of 2.5–3×10^6/mL using polyethylenimine (Polysciences) and harvest after 2d **48:00:00** expression, harvest and lyse cells with lysis buffer.

2 10m

Pellet the harvested cells at **500 x g** for **00:10:00** at **4 °C** , wash with PBS once, and then store at

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-80 °C .

3 Resuspend the cell pellet in Lysis Buffer, gentlely shake at 4 °C for 00:30:00 and clear it at 16.000 rpm at 4 °C for 00:30:00 . 1h

2- Protein Purification: 10h

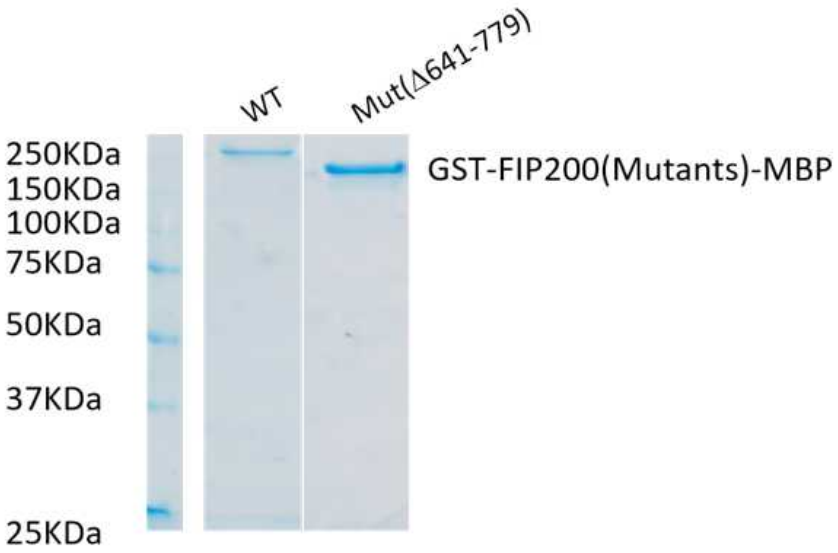
4 10h

Glutathione Sepharose 4B (GE Healthcare) followed by MBP-tag affinity purifications.

Incubate supernatant (added 6M NaCl to a final concentration of 0.5 M) with Glutathione Sepharose 4B resin at 4 °C with gentle shaking for 10:00:00 , apply to a gravity flow column, and wash extensively with Wash Buffer 1.

5 Elute protein of interest with Elution Buffer 1 and then further apply onto Amylose resin for a second step of affinity purification. Wash Amylose resin with Wash Buffer 2, and elute Protein of interest with Elution Buffer 2.

6 Pool peak fractions containing pure protein, concentrate, snap-freeze in liquid nitrogen and store at -80 °C .



Yield: about 0.2 mg per litter culture

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