

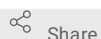


Jun 17, 2021

Expression and purification protocol of ULK1 Complex wt or K46I mutant

Xiaoshan Shi¹¹James Hurley Lab (UC, Berkeley)

1 Works for me



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ULK1 complex purification



Xiaoshan Shi

University of California, Berkeley, Shanghai Institute of Bi...

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ABSTRACT

The Protocol describes the expression and purification of ULK1 Complex wt or K46I mutant.

ATTACHMENTS

[Expression and purification protocol of ULK1 Complex wt or K46I mutant.pdf](#)

DOI

dx.doi.org/10.17504/protocols.io.bvn2n5ge

PROTOCOL CITATION

Xiaoshan Shi 2021. Expression and purification protocol of ULK1 Complex wt or K46I mutant.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.bvn2n5ge>

KEYWORDS

ULK1 Complex wt, K46I mutant

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CREATED

Jun 09, 2021

LAST MODIFIED

Jun 17, 2021

OWNERSHIP HISTORY

Jun 09, 2021  zahara93.zs

Jun 16, 2021  Xiaoshan Shi University of California, Berkeley

PROTOCOL INTEGER ID

50618

MATERIALS TEXT

Lysis Buffer: 50 mM Tris-HCl pH=7.4; 1% Triton X-100, 200 mM NaCl, 1 mM MgCl₂, 10% Glycerol, 1 mM TCEP, EDTA-free Protease Inhibitors (Roche).

Wash Buffer1: 50 mM Tris-HCl pH=7.4, 1% Triton X-100, 500 mM NaCl, 1 mM MgCl₂, 10% Glycerol, 1 mM TCEP;

Wash Buffer2: 20 mM Hepes pH=8, 200 mM NaCl, 2 mM MgCl₂, 1 mM TCEP;

Elution Buffer 1 (GST-tag): 50 mM Tris-HCl pH=7.4, 1% Triton X-100, 500 mM NaCl, 1 mM MgCl₂, 10% Glycerol, 1 mM TCEP, 50 mM Glutathione;

Elution Buffer 2 (Strep-tag): 50 mM Tris-HCl pH=7.4, 1% Triton X-100, 500 mM NaCl, 1 mM MgCl₂, 10% Glycerol, 1 mM TCEP, 10 mM desthiobiotin;

Elution Buffer 3 (MBP-tag): 20 mM Hepes pH=8, 200 mM NaCl, 2 mM MgCl₂, 1 mM TCEP, 50 mM Maltose;

Resin:

- Glutathione Sepharose 4B (GE Healthcare);
- Amylose resin (New England Biolabs);
- Strep-Tactin Sepharose (IBA Lifesciences).

SAFETY WARNINGS

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

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



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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: Synthetic codon-optimized DNAs encoding components Addgene 171411, 171412, 171414, 171416, 171417; backbone: all pCAG; resistance: MBP-TSF-TEVcs-ULK1 (Amp), GST-TEVcs-FIP200-MBP (Amp), ATG13 (Amp); GST-TEVcs-ATG101 (Amp); insert: *Homo sapiens* ULK1 Complex (ULK1, FIP200, ATG13, ATG101) (synthetic gene); tags & cleavage sites: MBP-TSF-TEVcs-ULK1, GST-TEVcs-ATG101, GST-TEVcs-FIP200-MBP, Ext coeff: 372900M⁻¹cm⁻¹, MW643kDa. FIP200/ATG13/ATG101 subcomplex and ULK1 are expressed and purified separately.

1- Protein expression:

2d 0h 10m

- 1 ULK1 and FIP200/ATG13/ATG101 are transfected and expressed separately. Transfect DNA in cells at a concentration of $2.5-3 \times 10^6$ /mL using polyethylenimine (Polysciences) and harvest after  **48:00:00** expression. Resuspend cells in PBS and harvest them by pelleting at  **500 x g** for  **00:10:00** at  **4 °C**, wash them with PBS once, and then store at  **-80 °C**.

- 2 Resuspend the cell pellets in Lysis Buffer, gently shake them at **4 °C** for **00:30:00** and clear them at **16.000 rpm** at **4 °C** for **00:30:00** . 1h

2- Protein Purification: 20h

- 3  14h

Glutathione Sepharose 4B and Strep-Tactin affinity purification followed by MBP-tag affinity purification.

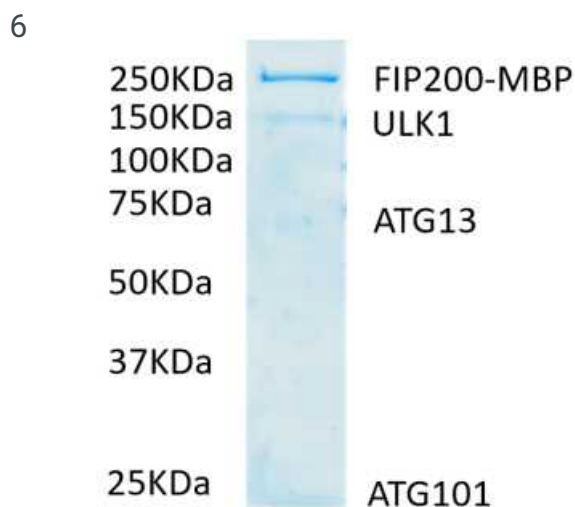
Incubate Supernatant (add 6M NaCl to a final concentration of 0.5M) respectively with either Glutathione resin or Strep-Tactin sepharose at **4 °C** with gentle shaking for **10:00:00** or **04:00:00** respectively, apply to a gravity column, and wash extensively with Wash Buffer1.

- 4  10h

Eluate Proteins of interest with Elution Buffer 1 or 2 respectively and then mix them for **Overnight** cleavage with Tobacco Etch Virus (TEV) protease at **4 °C** .

- 5 The mixture is subjected to a second step of affinity purification using the MBP tag.

Pass the eluted sample (with Elution Buffer 3) again through a Strep-Tactin Sepharose (IBA Lifesciences) column to clear the MBP-TSF tag from ULK1. Use final protein for assay immediately after purification.



Yield: about 0.1 mg per litter culture (600 ml FIP200/ATG13/ATG101 + 400 ml ULK1)

Protein stability: The complex aggregates easily and is degraded easily, so we suggest using fresh samples.