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Expression and purification protocol of ULK1 Complex wt or K46I mutant

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ULK1 complex purification


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DISCLAIMER

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

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PROTOCOL CITATION

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KEYWORDS

ULK1 Complex wt, K46I mutant

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MATERIALS TEXT

Lysis Buffer: 50 mM Tris-HCl 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 Buffer1: 50 mM Tris-HCl pH=7.4, 1% Triton X-100, 500 mM NaCl, 1 mM $MgCl_2$, 10% Glycerol, 1 mM TCEP;
Wash Buffer2: 20 mM Hepes pH=8, 200 mM NaCl, 2 mM $MgCl_2$,1 mM TCEP;
Elution Buffer 1 (GST-tag): 50 mM Tris-HCl pH=7.4, 1% Triton X-100, 500 mM NaCl, 1 mM $MgCl_2$, 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 $MgCl_2$, 10% Glycerol, 1 mM TCEP, 10 mM desthiobiotin;
Elution Buffer 3 (MBP-tag): 20 mM Hepes pH=8, 200 mM NaCl, 2 mM $MgCl_2$, 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).

DISCLAIMER:

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:372900$M^{-1}cm^{-1}$, 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 × 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** . 2d 0h 10m

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2 Resuspend the cell pellets in Lysis Buffer, gentlely 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.

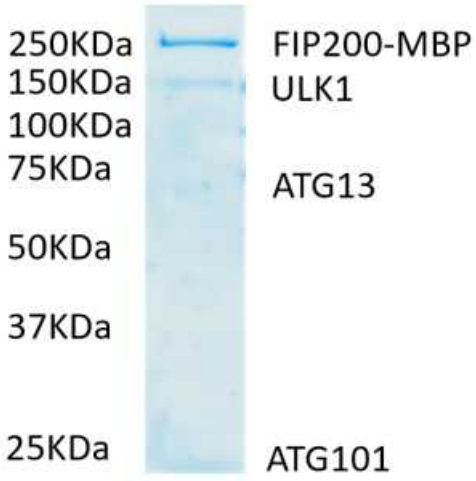
4 10h

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.

6



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.

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