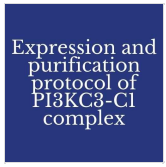

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Expression and purification protocol of PI3KC3-C1 complex

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ABSTRACT

This protocol outlines the procedure for expression and purification of the PI3KC3-C1 complex.

ATTACHMENTS

_ASAP_Team_Hurley_Hurley_lab_Chunmei_Chang_Exp_purif_protocol_PI3KC3.pdf

DOI

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PROTOCOL CITATION

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KEYWORDS

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MATERIALS TEXT

Transfection in HEK GnTI cells

Materials:

- polyethylenimine (PEI, Polysciences), make 1 mg/ml stock
- hybridoma media (Gibco)
- HEK293 expression freestyle media (Life Science Technology)
- all the DNA constructs

Protein purification

Materials:

- Glutathione Sepharose 4B (GE Healthcare)
- Strep-Tactin Sepharose (IBA)
- reduced glutathione (GSH, GE Healthcare)
- desthiobiotin (Sigma)
- protease inhibitor cocktail (Roche)
- TEV protease (1 mg/ml, homemade)

Buffers:

- lysis buffer: 50 mM HEPES pH 7.4, 1% Triton X-100, 200 mM NaCl, 1 mM MgCl2, 10% glycerol, and 1mM TCEP
- wash buffer: 50 mM HEPES pH 8.0, 200 mM NaCl, 1 mM MgCl2 and 1 mM TCEP
- GSH elution buffer: 50 mM HEPES pH 8.0, 200 mM NaCl, 50 mM GSH, 1 mM MgCl2 and 1 mM TCEP
- Strep elution buffer: 50 mM HEPES pH 8.0, 200 mM NaCl, 10 mM desthiobiotin, 1 mM MgCl2 and 1 mM TCEP
- Gel filtration buffer: 20 mM HEPES pH 8.0, 200 mM NaCl, 1 mM MgCl2 and 1 mM TCEP

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Plasmid amplification

1 Amplify the plasmid.

constructs: pCAG-VPS15, pCAG-TSF-VPS34, pCAG-TSF-BECN1, pCAG-GST-TEVs- A TG14

All of the constructs are Amp resistant and high copy, could be amplified in normal XL10 strain.

Transfection in HEK GnTI cells

2 Grow HEK293 GnTI suspension cells to 1.5-2 million cells/ml in the desired volume, **1 L cells** would be good for PI3KC3-C1 complex.

3 In warm hybridoma media, make 20 X solution of DNA (**20 µg/ml**) and PEI (**80 µg/ml**).

i.e. For a 1 L transfection add **4 mL PEI** to **50 mL Hybridoma media** and **1 mg DNA (in total)** in another **50 mL hybridoma media** . The ratio of the DNA is: 1.5 ATG14: 1.0 VPS34: 1.0 VPS15: 1.0 BECN1.

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4  30m

Add PEI to the DNA, mix together and incubate for a further **00:30:00** .

5 Add the DNA and PEI mixture to cells.

Harvest cells 2d 12h

6 Harvest cells after expression for **60:00:00** - **72:00:00** . 3d

7 

Spin down cells at **2000 rpm, 4°C, 00:20:00** .

8 

Wash the pellet with 1 X PBS once, freeze and store in **-80 °C** .

Protein purification 8h

9 

Lyse cells with the lysis buffer (**50 mL lysis buffer** for **1 L cell pellet**) supplemented with protease inhibitors. Clarify the lysate by centrifugation (**16000 rpm, 4°C, 01:00:00**).

10  4h

Incubate the supernatant with **2 mL Glutathione Sepharose 4B** at **4 °C** (or **Room temperature**) for **04:00:00** .

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11 

Apply the mixture to a gravity flow column and wash the resin extensively with wash buffer.

12 Elute the protein complex with **10 mL GSH elution buffer** .

13 

4h

Add **1 mg TEV protease** to eluted proteins and incubate at **4 °C** **Overnight** .

14 

Apply the TEV-treated complexes to a 1 ml Strep-Tactin Sepharose gravity flow column, wash the resin extensively with wash buffer.

15 Elute the protein complex with **5 mL Strep elution buffer** .

1 mL per fraction.

16 Apply the eluted fraction with highest concentration (or gently concentrate all the fractions to **1 mL**) to Superdex 6 column (10/300 Increase).

17 Collect the peak fractions and use freshly.

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