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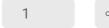
Purification of Human K560-GFP molecular motor

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Purification of Human K560-GFP molecular motor

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Mariusz Matyszewski

K560-GFP purification protocol from the Reck-Peterson Lab based on protocol from Nicholas et al. 2014. Edited for protocols.io by Andrea Dickey and Mariusz Matyszewski.

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All protein purification steps should be performed at 8 4 °C unless noted otherwise.

Materials: cOmplete EDTA-free protease inhibitor cocktail tablets lysozyme PMSF



1

Buffers:

Lysis buffer:

- [M]50 millimolar (mM) Tris pH 7.5
- [M]300 millimolar (mM) NaCl
- [M]5 millimolar (mM) MgCl2
- [M]0.2 Molarity (M) sucrose
- [M]1 millimolar (mM) DTT
- [M]0.1 millimolar (mM) Mg-ATP
- [M]0.5 millimolar (mM) Pefabloc

Wash buffer:

- [M]50 millimolar (mM) Tris pH 7.5
- [M]300 millimolar (mM) NaCl
- [M]5 millimolar (mM) MgCl2
- [M]0.2 Molarity (M) sucrose
- [M]10 millimolar (mM) imidazole
- [M]1 millimolar (mM) DTT
- [M]0.1 millimolar (mM) Mg-ATP
- [M]0.5 millimolar (mM) Pefabloc

Elution buffer:

- [M]50 millimolar (mM) Tris pH 8.0
- [M]300 millimolar (mM) NaCl
- [M]5 millimolar (mM) MgCl2
- [M]0.2 Molarity (M) sucrose
- [M]250 millimolar (mM) imidazole
- [M]5 millimolar (mM) bME
- [M]0.1 millimolar (mM) Mg-ATP

Storage buffer:

- [M]80 millimolar (mM) PIPES pH 7.0
- [M]2 millimolar (mM) MgCl2
- [M]1 millimolar (mM) EGTA
- [M]0.2 Molarity (M) sucrose
- [M]1 millimolar (mM) DTT
- [M]0.1 millimolar (mM) Mg-ATP



Glycerol cushion:

- [M]80 millimolar (mM) PIPES pH 7.0
- [M]2 millimolar (mM) MgCl2
- [M]1 millimolar (mM) EGTA
- [M]60 % volume glycerol
- [M]20 micromolar (μM) Taxol
- [M]1 millimolar (mM) DTT

BRB80:

- [M]80 millimolar (mM) PIPES pH 7.0
- [M]2 millimolar (mM) MgCl2
- [M]1 millimolar (mM) EGTA
- [M]300 millimolar (mM) KCl
- [M]7.5 millimolar (mM) Mg-ATP

Expression

- 1 pET17b-Kif5b(1-560)-GFP-His should be transformed into BL21(DE3)RIPL cells.
- 2 Make enough LB for at least **37.5** L of culture.
- 3 Grow an overnight starter culture.
- 4 Transfer starter culture into LB. Make sure to add proper antibiotics (Ampicillin for the plasmid, and Chloramphenicol for the cells we used).

Allow it to grow at \$\rightarrow\$200 rpm, 37°C until OD600 reaches 0.6-0.8.

- 5 Chill cells and incubator to § 18 °C
- 6 Add [M]0.5 millimolar (mM) IPTG and allow it to grow at \$\rightarrow\$200 rpm, 18°C, 18:00:00

7 Harvest and freeze cell pellets.

Purification 2h 35m

30m

- Resuspend T.5 L worth of pellets in 120 mL Lysis buffer supplemented with 1 cOmplete EDTA-free protease inhibitor cocktail tablet (Roche) per 50 mL of Lysis buffer. Also add M11 mg/mL lysozyme.

 Incubate 8 On ice for 00:30:00.
- 9 Lyse the resuspension by sonication.
- Add [M]0.5 millimolar (mM) PMSF to the sonicate, and clarify by centrifugation
 (340000 rcf, 4°C, 01:00:00 in Type 70 Ti rotor (Beckman).
 - 1h

1h

- 11 Incubate the supernatant with Ni-NTA agarose beads incubated with the **Wash buffer**.

 Nutate for **301:00:00**
- 12 Apply to gravity column and wash with **100 mL Wash buffer**.

- 5m
- Resuspend the beads in elution buffer, incubate for © 00:05:00 and elute in □0.5 mL increments.
- 14 Combine peak fractions and buffer exchange on PD-10 desalting column equilibrated with Storage buffer.
- Peak fractions of the motor solution were either flash-frozen at & -80 °C until further use or immediately subjected to microtubule bind and release purification (see next steps).

- A total of **1 mL** of motor solution from previous steps is used. Incubate with

 [M]1 millimolar (mM) AMP-PNP and [M]20 micromolar (μM) Taxol & On ice in the dark for © 00:05:00 and subsequently warm to & Room temperature.
- Polymerize bovine brain tubulin (about © 00:30:00 at & 37 °C) and centrifuge (TLA120.2 rotor at **380000 rpm, 00:12:00** at & Room temperature) through a glycerol cushion. Resuspend the pellet.
- Incubate the microtubule solution with the resuspended microtubules in the **dark** for © **00:15:00** at § **Room temperature**.
- Put the incubated solution on top of **glycerol cushion** and centrifuge in a TLA120.2 rotor at **8 Room temperature**.
- 20 Wash the final pellet with BRB80 and incubate in \blacksquare 100 μ L of release buffer for \bigcirc 00:05:00 at & Room temperature .
- Centrifuge the kinesin solution **372000 rpm, 00:07:00** in TLA100 rotor at **8 Room temperature** .
- Collect the supernatant and supplement with [M]660 millimolar (mM) sucrose and flash freeze.

A typical kinesin prep in the lab yielded [M10.5 micromolar (μ M) to [M1.5 micromolar (μ M) K560-GFP dimer.