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Purification of Human K560-GFP molecular motor

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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 **4 °C** unless noted otherwise.

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

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

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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 7.5 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 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 200 rpm, 18°C, 18:00:00

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7 Harvest and freeze cell pellets.

Purification 2h 35m

8 Resuspend **7.5 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** . 30m
Also add **1 mg/mL lysozyme** .
Incubate **On ice** for **00:30:00** .

9 Lyse the resuspension by sonication.

10 Add **0.5 millimolar (mM) PMSF** to the sonicate, and clarify by centrifugation **40000 rcf, 4°C, 01:00:00** in Type 70 Ti rotor (Beckman). 1h

11 Incubate the supernatant with Ni-NTA agarose beads incubated with the **Wash buffer**. 1h
Nutate for **01:00:00**

12 Apply to gravity column and wash with **100 mL Wash buffer** .

13 Resuspend the beads in elution buffer, incubate for **00:05:00** and elute in **0.5 mL** increments. 5m

14 Combine peak fractions and buffer exchange on PD-10 desalting column equilibrated with **Storage buffer**.

15 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).

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Microtubule bind and release purification 44m

16 A total of **1 mL** of motor solution from previous steps is used. Incubate with **1 millimolar (mM) AMP-PNP** and **20 micromolar (µM) Taxol** **On ice** in the **dark** for **00:05:00** and subsequently warm to **Room temperature** . 5m

17 Polymerize bovine brain tubulin (about **00:30:00** at **37 °C**) and centrifuge (TLA120.2 rotor at **80000 rpm, 00:12:00** at **Room temperature**) through a **glycerol cushion**. Resuspend the pellet. 42m

18 Incubate the microtubule solution with the resuspended microtubules in the **dark** for **00:15:00** at **Room temperature** . 15m

19 Put the incubated solution on top of **glycerol cushion** and centrifuge in a TLA120.2 rotor at **80000 rpm, 00:12:00** at **Room temperature** . 12m

20 Wash the final pellet with **BRB80** and incubate in **100 µL of release buffer** for **00:05:00** at **Room temperature** . 5m

21 Centrifuge the kinesin solution **72000 rpm, 00:07:00** in TLA100 rotor at **Room temperature** . 7m

22 Collect the supernatant and supplement with **660 millimolar (mM) sucrose** and flash freeze.

A typical kinesin prep in the lab yielded **0.5 micromolar (µM)** to **1.5 micromolar (µM)** K560-GFP dimer.

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