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Purification of Human K560-GFP motor (without B+R)

Forked from Purification of Human K560-GFP molecular motor

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We use this protocol and it's working

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Abstract

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 in 2022 and modified in 2024 for - bind and release by Kavi Rangan and Katy Surridge.

Guidelines

All protein purification steps should be performed at 4 °C unless noted otherwise. From Ni-NTA step onwards, purification steps should be completed in the dark.

Materials

Materials:
cOmplete EDTA-free protease inhibitor cocktail tablets (ROCHE)
lysozyme

Buffers:
Lysis buffer (make 100 mL):

- [M] 50 millimolar (mM) Tris pH 7.5
- [M] 250 millimolar (mM) NaCl
- [M] 1 millimolar (mM) MgCl2
- [M] 20 millimolar (mM) Imidazole
- [M] 10 millimolar (mM) BME
- [M] 0.5 millimolar (mM) Mg-ATP
- [M] 1 millimolar (mM) Pefabloc

Wash buffer (make 500 mL):

- [M] 50 millimolar (mM) Tris pH 7.5
- [M] 250 millimolar (mM) NaCl
- [M] 1 millimolar (mM) MgCl2
- [M] 20 millimolar (mM) imidazole
- [M] 10 millimolar (mM) BME
- [M] 0.5 millimolar (mM) Mg-ATP
- [M] 1 millimolar (mM) Pefabloc

Elution buffer (make 100 mL):

- [M] 50 millimolar (mM) Tris pH 8.0
- [M] 250 millimolar (mM) NaCl
- [M] 1 millimolar (mM) MgCl2
- [M] 250 millimolar (mM) imidazole
- [M] 10 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] 10 % volume sucrose
- [M] 0.1 millimolar (mM) DTT
- [M] 0.1 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 2 L of culture.

3 Grow an overnight starter culture.

4 Transfer starter culture into LB. Make sure to add proper antibiotics
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] 0.75 millimolar (mM) IPTG and allow it to grow at 200 rpm, 18°C, 16:00:00

7 Harvest pellets by centrifugation, wash in ice cold PBS and freeze in liquid nitrogen until use.

Purification

2h 35m

8 Resuspend 2 L worth of pellets in 40 mL Lysis buffer supplemented with 1 cOmplete EDTA-free protease inhibitor cocktail tablet (Roche) per 40 mL of Lysis buffer . 30m
Also add 1 mL of [M] 50 mg/mL lysozyme . Vortex vigorously to resuspend.
Incubate On ice for 00:30:00 .

9 Lyse the resuspension by sonication on ice with pulse cycles 00:00:05 on and 00:00:20 rest. 25s

10 Clarify by centrifugation 92600 x g, 4°C, 00:30:00 in Type 70 Ti rotor (Beckman). 30m

11 During centrifugation, wash each 4 mL Ni-NTA agarose slurry with 20 mL **Wash buffer**.

12 Incubate the supernatant with Ni-NTA agarose beads washed with the **Wash buffer**. Nutate for 01:00:00 at 4 °C in the dark (from this step onwards, keep in dark as much as possible). 1h

13 Apply to gravity column and wash 4 times with 10 mL Wash buffer each time.

14 Resuspend the beads in 1 mL elution buffer, incubate for 00:02:00 and elute in 0.5 mL increments. Repeat elution step up to 5 times (following green GFP signal) 2m

15 Combine peak fractions and buffer exchange on PD-10 desalting column equilibrated with **Storage buffer (BRB80)**.

16 Peak fractions of the motor solution were flash-frozen at -80 °C until further use. Bind and release protocol could be used at this stage, see: **dx.doi.org/10.17504/protocols.io.bp2l61xrdvqe/v1**