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

 Forked from [Purification of Human K560-GFP molecular motor](#)

Purification of
Human K560-GFP
molecular motor

 [Science Advances](#)

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Protocol status: Working

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  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 . Also add  1 mL of  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.
- 10 Clarify by centrifugation  92600 x g, 4°C, 00:30:00 in Type 70 Ti rotor (Beckman).

30m

25s

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