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Rab8a expression and purification

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Recombinant Rab8a expression and purification protocol as used by the Leschziner and Reck-Peterson Labs.

Original protocol by David Snead. Adapted for protocols.io by Mariusz Matyszewski.

Current version as used in Snead, Matyszewski, Dickey et al. 2022.

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Materials:
Ni-NTA beads
IgG beads
Purified TEV protease

Equipment:
FPLC
Ultracentrifuge
Sonicator

Buffers:

Lysis Buffer (make **150 mL**):

- **50 millimolar (mM) HEPES pH 7.4**
- **200 millimolar (mM) NaCl**
- **10 % volume Glycerol**
- **5 millimolar (mM) MgCl2**
- **2 millimolar (mM) DTT**
- **0.5 millimolar (mM) PefaBloc**
- 1 cOmplete Protease Inhibitor Cocktail tablet per **50 mL** (3 for suggested volume in this prep)

Wash Buffer (make **750 mL**):

- **50 millimolar (mM) HEPES pH 7.4**
- **150 millimolar (mM) NaCl**
- **5 % volume Glycerol**
- **5 millimolar (mM) MgCl2**
- **1 millimolar (mM) DTT**

Elution Buffer (make **100 mL**):

- **50 millimolar (mM) HEPES pH 7.4**
- **150 millimolar (mM) NaCl**
- **5 % volume Glycerol**
- **5 millimolar (mM) MgCl2**
- **1 millimolar (mM) DTT**
- **300 millimolar (mM) Imidazole pH 8.0**

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TEV Buffer (make **200 mL** ; will also need a little bit of extra TEV buffer with **10 % volume Glycerol** for storage):

- **50 millimolar (mM) HEPES pH 7.4**
- **200 millimolar (mM) NaCl**
- **5 % volume Glycerol**
- **5 millimolar (mM) MgCl2**
- **1 millimolar (mM) DTT**

S200 Buffer (make **250 mL**):

- **50 millimolar (mM) HEPES pH 7.4**
- **200 millimolar (mM) NaCl**
- **1 % volume Glycerol**
- **5 millimolar (mM) MgCl2**
- **1 millimolar (mM) DTT**

Expression 1h 6m

1 Transform n-terminally His6-ZZ tagged Rab8a (pET28a backbone) into BL21(DE3) E. coli cells.

> BL21-CodonPlus (DE3)-RIPL have also been used successfully. Please adjust the antibiotics used based on the cells used.
> pET28a backbone comes with Kanamycin resistance

2 Make fresh LB media. This protocol assumes **4 L (2L per flask)** are made. Scale accordingly.

3 Grow an overnight culture in LB media with **50 µg/mL Kanamycin**
Make **50 mL** . This is enough for the main 4L growth.

4 Add the overnight culture into main LB flasks with antibiotic present (**50 µg/mL Kanamycin**). Use 100x dilution (**10 mL per 1L**).

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5 Let it growth in a shaker at **200 rpm, 37°C** until OD600 of 0.4-0.5 is reached.

6 Chill the cells and set the shaker to **18 °C** . We chill the cells in the cold room at **4 °C** for **01:00:00** 1h

7 Add IPTG at **0.5 millimolar (mM) final concentration** into the chilled culture.

Higher IPTG concentrations can be used if needed.

8 Let it grow in a shaker at **200 rpm, 18°C, 18:00:00**

9 Harvest the cells. We spun them down using a JLA 9.1000 rotor at **6000 rpm, 4°C, 00:06:00** in 1L Beckman bottles. We then resuspended the cells in **15-30 mL LB** and transferred them to 50 mL Falcon tubes (1 tube per 2L harvested). Flash frozen with liquid nitrogen and stored in **-80 °C** freezer. 6m

Purification: Day 1 2h

10 Resuspend the cell pellet in **lysis buffer**. Add about **40 mg Lysozyme** and incubate **00:30:00** at **On ice** 30m

11 Lyse the cells by sonication **On ice** .

12 Ultracentrigation in Ti-70 rotor **30000 rpm, 4°C, 00:30:00** 30m

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Done using ultracentrifugation in our lab, but regular centrifugation should work too. Adjust accordingly.

12.1 While centrifuging, prepare **2 mL Ni-NTA beads** by equilibrating with **lysis buffer**.

13 Once centrifugation is done, add the beads to the supernatant. Let it nutate for **01:00:00** 1h **4 °C** .

13.1 Prepare **Wash and Elution buffers** while waiting.

14 Apply beads to a gravity column and wash with **250 mL Wash buffer**

15 Elute with **40 mL Elution buffer** . Best to resuspend in **20 mL** , elute, resuspend again, and finish elution.

16 Equilibrate IgG beads with **Wash Buffer**.

17 Dilute the eluted protein to **90 mL Wash Buffer** . Split in 2 (**45 mL each**). Add **2 mL IgG beads** to each.

18 Nutate at **4 °C** for 2-3 hours.

19 Apply to gravity column and wash with **250 mL Wash buffer**

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20 Make **TEV buffer** and transfer beads with **10 mL TEV Buffer** into 15 mL tubes.

21 Add **400 µL** **1.3 mg/mL** TEV protease and incubate **Overnight** at **4 °C**

Purification: Day 2

22 Equilibrate Ni-NTA beads with **TEV buffer** in gravity column.

23 Pour cleaved protein onto the beads. Collect flowthrough.

24 Wash with leftover **TEV buffer**. Collect flowthrough as well.

25 Combine all flowthroughs and concentrate to **1 mL**

26 Equilibrate S200I column with **S200 buffer**.

> Other SEC columns can be used instead. S75 should have good separation too.

27 Apply concentrated protein to the S200I column and run S200I program.

28 Concentrate protein and do buffer exchange with **TEV buffer modified with 10% glycerol.**

29 Concentrate protein, quantify, and flash freeze for **-80 °C** storage.

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