

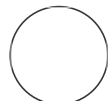


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## 🌐 Cryo-EM sample preparation for PI3KC3-C1~RAB1A complex

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### ABSTRACT

This protocol details cryo-EM sample preparation for PI3KC3-C1~RAB1A complex.

### ATTACHMENTS

[852-2199.pdf](#)

### MATERIALS

#### Materials

- RAB1A(Q70L) protein
- EDTA
- GTP
- PD-10 column
- HEPES pH 7.5
- NaCl
- MgCl<sub>2</sub>
- TCEP
- PI3KC3-C1 protein

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**protocols.io**

<https://protocols.io/view/cryo-em-sample-preparation-for-pi3kc3-c1-rab1a-com-c2pcydw>

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**Protocol status:** Working  
We use this protocol and it's working

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**PROTOCOL integer ID:**  
88516

## GTP Loading of RAB1A

1h 20m

- 1 Strip the protein of its bound divalent cations by incubating RAB1A(Q70L) with  EDTA at  for .



30m

- 2 Add a large molar excess () of GTP to the protein.

- 3 Incubate the mixture for an additional  to allow GTP loading.



30m

- 4 Quench the reaction by adding   $\text{MgCl}_2$  at  for .



20m

## Desalting of GTP-loaded RAB1A

- 5 Purify the GTP-loaded RAB1A through a PD-10 column equilibrated with a final buffer containing  HEPES ,   $\text{NaCl}$ ,   $\text{MgCl}_2$ , and  TCEP.

## Preparation of PI3KC3-C1~RAB1A Complex

1h

- 6 Prepare a 1:20 molar ratio of PI3KC3-C1 to RAB1A ( PI3KC3-C1:  RAB1A).

- 7 Incubate the mixture for  at  to allow complex formation.



1h