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

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Protocol status: Working

We use this protocol and it's working

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Abstract

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

Attachments



[852-2199.pdf](#)

28KB

Materials

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- RAB1A(Q70L) protein
- EDTA
- GTP
- PD-10 column
- HEPES pH 7.5
- NaCl
- MgCl₂
- TCEP
- PI3KC3-C1 protein



GTP Loading of RAB1A

1h 20m

1 Strip the protein of its bound divalent cations by incubating RAB1A(Q70L) with **5 millimolar (mM)** EDTA at **Room temperature** for **00:30:00**.

30m



2 Add a large molar excess (**50 millimolar (mM)**) of GTP to the protein.

3 Incubate the mixture for an additional **00:30:00** to allow GTP loading.

30m



4 Quench the reaction by adding **20 millimolar (mM)** MgCl_2 at **Room temperature** for **00:20:00**.

20m



Desalting of GTP-loaded RAB1A

5 Purify the GTP-loaded RAB1A through a PD-10 column equilibrated with a final buffer containing **25 millimolar (mM)** HEPES **pH 7.5**, **150 millimolar (mM)** NaCl, **2 millimolar (mM)** MgCl_2 , and **25 millimolar (mM)** TCEP.

Preparation of PI3KC3-C1~RAB1A Complex

1h

6 Prepare a 1:20 molar ratio of PI3KC3-C1 to RAB1A (**0.55 micromolar (μM)** PI3KC3-C1: **11 micromolar (μM)** RAB1A).

7 Incubate the mixture for **01:00:00** at **Room temperature** to allow complex formation.

1h

