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ADP Glo Protocol

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

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Abstract

Protocol for ADP Glo (Promega) assay to measure the catalytic activity of the lipid kinase VPS34 on small unilammelar vesicles.

Attachments

PDF

852-2202.pdf

34KB

Materials

Materials

- Small unilamellar vesicles (SUVs) composed of

	A
	40% dioleoyl phosphatidylcholine (DOPC)
	20% dioleoyl phosphatidylethanolamine
	20% dioleoyl phosphatidylserine
	20% liver phosphatidylinositol

All lipids from Avanti Polar Lipids.

- HEPES pH 7.5
- NaCl
- $MgCl_2$
- $MnCl_2$
- TCEP
- ATP
- PI3KC3-C1
- ADP-Glo ATP Depletion Reagent (Promega)

Equipment

- Glass tube
- N2 stream source
- Vacuum dessicator
- Benchtop centrifuge
- Sonicator with a small probe tip
- Water bath at 37°C
- Liquid nitrogen

Preparation of Small Unilamellar Vesicles (SUVs)

15m

1 Dehydrate 0.4 mg of the lipid mixture (40% DOPC, 20% DOPE, 20% DOPS, 20% liver phosphatidylinositol, Avanti Polar Lipids) in a glass tube using a gentle N2 stream.

2 Allow the lipids to dry Overnight in a vacuum desiccator to remove excess chloroform.

3 Rehydrate the dried lipids by adding [M] 25 millimolar (mM) HEPES pH 7.5 and [M] 200 millimolar (mM) NaCl.

4 Vortex the mixture to resolubilize the lipids.

5 Perform 10 cycles of freeze-thaw by immersing the lipids in liquid nitrogen for a few seconds and then in a 37 °C water bath.

6 Sonicate the lipids using a small probe tip sonicator at 50% power in 2-second on/off cycles for a total of 00:15:00 On ice and a water mixture. 15m

Preparation of Buffers

7 Prepare a kinase dilution buffer.

Kinase dilution buffer

	A	B
	HEPES pH 7.5	25 mM
	NaCl	200 mM
	$MgCl_2$	10 mM
	$MnCl_2$	1 mM
	TCEP	2 mM

8 Prepare a 2x lipid dilution buffer.

2x lipid dilution buffer

	A	B
	HEPES pH 7.5	25 mM
	NaCl	200 mM
	$MgCl_2$	20 mM
	$MnCl_2$	2 mM
	TCEP	4 mM

ADP-Glo assay

1h 40m

9 In a reaction tube, combine the following components:

	A	B
	50 nM PI3KC3-C1	9 µL
	0.1 mg/mL SUVs in the 2x lipid dilution buffer	9 µL
	500 µM ATP in the same buffer	2 µL

10 Mix the components gently.

11 Allow the reaction to proceed for 01:00:00 at Room temperature .

1h

12 After the incubation, add 20 µL of the ADP-Glo ATP depletion reagent to each reaction tube.

13 Incubate the reaction tubes with the ADP-Glo ATP depletion reagent for 00:40:00 at Room temperature .

40m

14 Measure the luminescent output for each condition tested using a luminometer or a plate reader.