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Tethering and lipid transfer assay

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

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Abstract

This protocol describes the process of liposome tethering and lipid transfer.

Materials

Reaction Mix

	A	B
	Donor liposomes (of 0.5 mg/mL total lipids)	2.5 µL
	Acceptor liposomes (of 0.5 mg/mL total lipids)	2.5 µL
	Proteins (final concentrations as per figure legends):	
	ATG2A	250 nM
	WIPI4	100 nM
	LC3B	100 nM

Equipment

Glass-bottom 384-well microplates — NAME

SENSOPLATE — BRAND

781892 — SKU

https://shop.gbo.com/en/india/products/bioscience/microplates/sensoplate-glass-bottom-plates/384-well-sensoplate/781892.html — LINK

Plate Setup and Reaction Conditions

1 Perform reactions in glass-bottom 384-well microplates (Greiner Bio-One, Cat# 781892).

2 Carry out all assays at Room temperature .

Reaction Mix

3 Each 50 µL reaction (in SEC300 buffer) includes:

	A	B
	Donor liposomes (of 0.5 mg/mL total lipids)	2.5 µL
	Acceptor liposomes (of 0.5 mg/mL total lipids)	2.5 µL
	Proteins (final concentrations as per figure legends):	
	ATG2A	250 nM
	WIPI4	100 nM
	LC3B	100 nM

NBD Fluorescence Measurement (Lipid Transfer)

2h 20m 20s

4 Use a Tecan SPARK Multimode Microplate Reader (RRID:SCR_021897).

5 Record NBD fluorescence:

1h 30m 20s

- Excitation: 485 nm
- Emission: 535 nm
- Measurement interval: every 00:00:20
- Duration: 01:30:00

6 Add 0.5% (v/v) DDM to each well to fully solubilize lipids.

- 2.5 µL of 10% (v/v) DDM to 50 µL reactions.

7 Measure maximum NBD fluorescence 00:50:00 after DDM addition. 50m

8 Normalize fluorescence:
- Subtract the initial fluorescence value.
- Divide by the maximal DDM-treated fluorescence.

Liposome Tethering (Optional Parallel Readout)

9 Measure absorbance at 405 nm every 00:00:20 in the same wells.

10 This quantifies vesicle clustering (tethering) under identical conditions using the same plate reader.