

NavAb Proton Flux Assay Protocol

Making the Liposome

1. Dissolve POPE/POPG (3:1, w/w) in a buffer containing 20mM Hepes, 150 mM KCl, 1% LMNG, NMDG adjusted pH 7.5, with final concentration of 5 mg/ml (0.8 ml tube with 4mg of total lipids). Store at -80 Celsius.
2. Thaw the lipid stock in RT, add 3.2 ul TCEP (0.5M=500mM stock) to reach final concentration of 2 mM. Mix with WT NavAb protein at a ratio of 1:8000 (w/w) equivalent to 1ul of NavAb for 800ul of total lipids.
 - a. Incubate at 4 Celsius for 20 minutes.
3. Add 0.3g of Bio-beads SM2 to remove detergent overnight.
4. Next day use a 1 ml syringe and 23g needle to suck the liposome out and discard the Bio-beads SM2. Ensure the flat end of the syringe is placed against the wall of the collection tube to avoid sucking in Bio-beads.
5. Store the formed liposomes at -80 Celsius for later use in the liposome flux Assay (LFA).

Liposome Flux Assay

1. Obtain assay plates. Wells that have not been used have no tape on them and can be used for the LFA.
2. Solutions used:
 - a. ACMA stock (1mM in DMSO), working conc of 2uM (500x)
 - b. Valinomycin stock (4.5uM in ethanol), working conc of 0.045uM (100x)-Not accurate
 - c. CCCP stock (0.1 mM stock in DMSO), working concentration of 1 uM (100x)
3. Compounds are added in this format:
 - a. Dilute KCL in 150mM NMDG/20mM Hepes (pH 7.4) and add to the assay plate
 - i. Alternatively, just add NMDG/Hepes to the assay plate
 - b. Add ACMA to the constituted liposome
 - c. Add the liposome/ACMA to the compounds added from (a) above.
 - d. Add Valinomycin to trigger K⁺ efflux to establish a potential difference (Nernst Potential) that will trigger H⁺ influx into the cell.
 - e. For positive control add CCCP to trigger H⁺ influx in a NavAb independent manner.
4. Positive Control:
 - a. Add CCCP before pause > add Valinomycin after pause
5. Negative Control:
 - a. No Valinomycin before pause > Optionally add CCCP after pause
 - b. This result explained by some K⁺ leakage that leads to H⁺ influx
6. Follow the Excel sheet for calculations (Working on this).