vcCNT Vesicle Flux Assay

1. Freeze/thaw vesicles in liquid nitrogen 3 times.
2. Extrude vesicles through 1.0 μm filter using the Avanti Mini Extruder, passing through filter 15 times.
3. Dilute vesicles 20-fold into reaction buffer. (For typical experiment with inward sodium gradient, use 100 mM NaCl, 200 mM KCl, 20 mM HEPES pH 7.4. NaCl can be replaced with ChoCl to adjust or remove sodium gradient.)
4. Aliquot 200 μL/reaction of diluted vesicles into individual wells of 96-well block.
5. Make master mixes of radiolabeled nucleoside and valinomycin in PCR tubes. (Typically use 20 μL of stock radiolabeled nucleoside per reaction and 10 μL of 20 μM valinomycin per reaction for final concentration of 1 μM valinomycin. Master mix should contain a total volume of 1.5 x total number of reactions.)
6. Incubate everything (block + PCR tubes) at 30 °C for ~10 min.
7. Prepare harvester and 1-μm GF/B filter paper (Whatman) by washing system and filter paper with wash buffer (100 mM ChoCl, 200 mM KCl, 20 mM HEPES pH 7.4).
8. Add and mix by pipetting 30 μL of nucleoside/valinomycin mixture to each well at specified time points using multi-channel pipette.
9. Harvest at end of time course by aspirating with harvester and washing filter paper 4 times with wash buffer.
10. Transfer the cut circles of filter paper (harvester makes these cuts into the filter paper) to individual scintillation vials using tweezers and add 5 mL of Lefko-Fluor scintillation fluid.
11. Count by scintillation using ^{3}H protocol the following day.