SLO exp. Protocol

1. Make KOAc buffer (pH7.4)

115 mM KOAc
25 mM HEPES
2.5 mM MgCl2

2. Activate SLO.
a. In 1ml KOAc buffer, add 6ul (1mg/ml stock) SLO, final conc. is 6ug/ul. (Note: you might need to titrate the concentration depending on your experiments.)
b. Add DTT to KoAc buffer, final conc. is 10mM
c. Activate SLO at 37C for 30mins and then keep it at 4C until used for permeabilization.
d. Add CaCl2 to 1mM to the activated SLO.

3. Washed cells are spin down at 4C and resuspended in activated SLO, incubate at 4C for 15mins.

4. After incubation, cells are spin down and washed with TM buffer to remove excess SLO, and then resuspended in TM buffer. Incubate the cells in TM buffer for 30mins at 37C.

TM Buffer:
(KOAc buffer containing 1mM DTT, 5mM EGTA)

5. After incubation, spin down cells and wash with ice cold 1XPBS.

6. Cytospin to load the cells onto slides.

7. Stain with antibodies.