**Bead assay in 96 well plate**

Prior to the assay, lay out test compounds (or compounds of different concentration) in test wells of the plate. Control wells receive the same amount of solvent (DMSO, isopropanol, or methanol/water 1:1) as test wells.

**Compound incubation**

Obtain TALL cells, 27 million (27E6) cells for one 96 wp, wash in normal Ringer’s (NR) saline solution once, and re-suspend in 11 milliliter NR, aliquot 100 microliter to each well using multi-channel pipettor. Cells are 250,000 (0.25E6) in 100µl system per well.

Mix with compounds extensively using multi-channel pipettor. 30 cycles of mixing at 100µl volume.

Incubate the plate in 37 Celcius degree for 30 minute.

**LAMP assay incubation**

After compound incubation, prepare LAMP assay components (see below\*), mix and add 10µl to each well:

CD8

10µl CD8 beads after wash

5µl LAMP antibody

4µl DMSO

80µl NR

100µl total

10µl each well into control wells

CD3 (need to scale up for 96-wp, prepare 11X as this formula)

2.5µl CD3 beads after wash

5µl LAMP antibody

4µl DMSO

90µl NR

100µl total

10µl each well into control wells and all test wells

CD3 (11X)

27.5µl CD3 beads after wash

55µl LAMP antibody

44µl DMSO

974µl NR

1100µl total

170µl aliquot into 6 wells

10µl each well using 6 channel pippetting

CD3 superstim

2.5µl CD3 beads after wash

5µl LAMP antibody

2µl TG@1mM

2µl PMA@50µM

90µl NR

100µl total

10µl each well into control wells

Mix cells with LAMP assay components extensively using multi-channel pipettor. 30 cycles of mixing at 100µl volume.

Seal the plate with parafilm.

Incubate the plate in room temperature for 60 minute with constant rotation.

\*Test conditions in each well:

CD8 bead (4E8 beads/ml, 1:100 dilution), 400000 beads vs 250000 cells=1.6

CD3 bead (4E8 beads/ml, 1:400 dilution), 100000 beads vs 250000 cells=0.4

LAMP antibody (0.1mg/ml, 1:200 dilution), 0.5µg/ml in 100µl

TG (1mM, 1:500 dilution), 2µM in 100µl

PMA (50µM, 1:500 dilution), 100nM in 100µl

DMSO from LAMP assay=0.4%

**Cell fixation and flow cytometry**

After LAMP assay incubation, add 100µl 2%PFA into each well, mix, and transfer each well content to microtiter tubes in 96 tube rack. Place the microtiter tubes in flow cytometer tubes for sample analysis.

Store the cell samples at 4 Celcius degree until flow cytometer analysis.