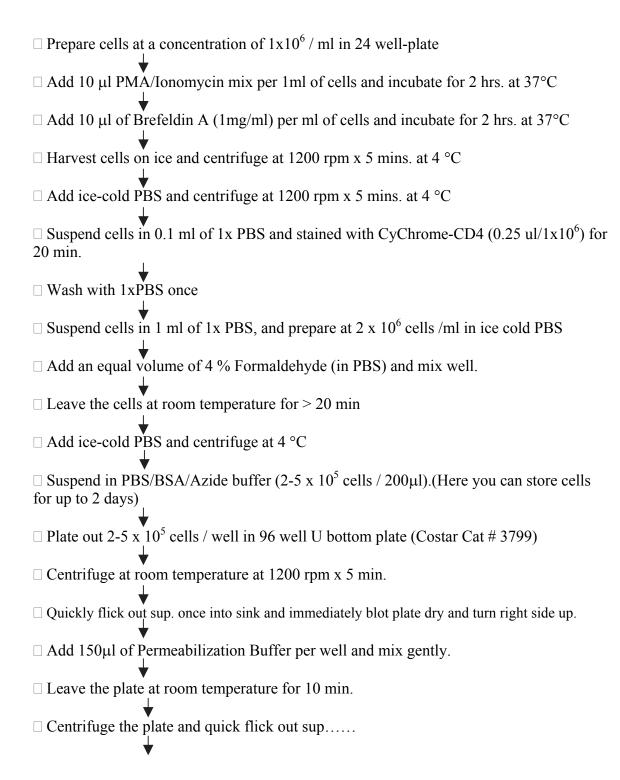
## **Intracellular cytokine staining Protocol**



□ Add 25 μl of Permeabilization Buffer containing antibodies.

1: no antibodies

2: FITC-isotype ctr, PE-isotype ctr

3: FITC-IFNg (0.25 ul), PE-IL-4 (0.25 ul)

4: FITC-IL-2 (), PE-TNFa ()

5: FITC-IL-10 (), PE-IL-5 ()

□ incubate for 30 min at room temperature in the dark

□ Add 150 μl of Permeabilization Buffer / well

Centrifuge
□ Flick out sup...... and add 150 μl of Permeabilization Buffer (no Saponin) / well

Centrifuge
□ Flick out sup...... and add 150 μl of PBS/BSA/Azide Buffer (no Saponin) / well

Flick out sup...... and add 150 μl of PBS/BSA/Azide Buffer / well

Flick out sup...... and add 150 μl of PBS/BSA/Azide Buffer / well

# **Regents and solutions**

#### PMA/Ionomycin mix

PMA: Sigma #P-8139

Ionomycin: Calbiochem #407952

PMA stock :5 mg/ml in DMSO, aliquots of 50 ul, store at -80C)

Ionomycin stock: 1mM in DMSO

Dilute 25 ul of PMA in 2.5 ml of culture media (10% FCS) to make a stock of 50 ug/ml (**diluted PMA**)

Mix 20 ul of diluted PMA (final 5 ug/ml)

13.5 ul of ionomycin (final 50 ug/ml)

166.5 ul of culture media

add 10 ul of PMA/Ionomycin mix per 1 ml of cells (Final concentrations of PMA and Ionomycin in media will be 50 ng/ml and 500 ng/ml, respectively).

### Brefeldin A (BFA)

Dissolve 1 mg of BFA (Sigma #) in 1 ml of 100% EtOH, and store at -80C. Add 10 ul of BFA per 1 ml of cells

#### PBS/BSA/Azide buffer

1 liter of PBS pH7.4 650 ul of 10% Azide

Layer 5g of BSA on top of the liquid mixture. Do not shake the bottle. Allow BSA to dissolve at room temperature, without stirring. Sterile filter the mixture. Store at 4C.

## 10 % Saponin:

Mix 5g Saponin (Sigma #S-7900) 50 ml PBS pH7.4

Place at 37C until the saponin has dissolved completely.

Filter the solution through a 0.22 micron filter

Store the solution at 4C.

### Permeabilization Buffer

Mix 5 ml 10% Saponin 95 ml PBS/BSA/Azide buffer