131110 **FLAG intracellular staining**

Originally developed by Hodaka Fujii on September 18, 2012

Modified by Hodaka Fujii on November 10, 2013

**Reagents**

eBioscience

Fixation/Permeabilization Concentrate

Fixation/Permeabilizaion Diluent

10 x Permeabilization buffer

Sigma

anti-FLAG M2-FITC F4049

- Suspend 1 x 106 cells in 700 µl of freshly made Fixation/Permeabilization working solution.

Fixation/Permeabilization Concentrate **0.175 ml**

Fixation/Permeabilizaion Diluent **0.525 ml**

- 4°C for 30 min - 18 hr.

- Wash (4,000 rpm, 1 min) with 700 µl and 1 ml of 1 x Permeabilization buffer

(total x 2).

10 x Permeabilization buffer **0.5 ml**

DDW **4.5 ml**

- Suspend in 50 µl of diluted Ab (1/500 dilution).

anti-FLAG M2-FITC (1.1 µg/µl)  **1 µl**

1 x Permeabilization buffer **499 µl**

- 4°C, 30 min.

- Wash with 1 ml of 1 x Permeabilization buffer x 2.

- Suspend in 500 µl of FACS buffer to analyze.