Cell Surface Expression Assay

December, 2008

Version 1.0

Summary:

This protocol is used by JCIMPT to determine the cell surface expression of FLAG tagged constructs. Production of structure-grade mammalian membrane proteins in substantial quantities has been hindered by a lack of methods for effectively profiling multiple constructs expression in higher eukaryotic systems such as insect or mammalian cells. To address this problem, a specialized small-scale eukaryotic expression platform by Thomson Instrument Company (Vertiga-IM) was developed and used in tandem with a Guava EasyCyte microcapillary 96-well cytometer to monitor cell density and health and evaluate membrane protein expression. The reference (Hanson et al. 2007) given below provides detailed description of work done to develop a construct profiling method that uses this protocol.

Materials:

1. **Reagents:**
    a. Cell strain expressing the desired construct.
    b. Positive control.
    c. Anti-FLAG M2 Monoclonal Antibody (SIGMA, catalog number F-3165).
    d. Zenon Alexa Fluor 488 Mouse IgG_1 Labeling Kit (Invitrogen, catalog number Z25002)

2. **Equipment:**
    a. Guava EasyCyte flow cytometer (Guava Technologies)

Step by step methodology

a. Prepare a 0.2 µg/µL antibody mix in Zenon Alexa Fluor 488. Incubate 5 minutes at room temperature. Dilute 1:10 with Tris Buffered Saline (TBS) containing 4% BSA (final concentration of antibody = 0.02 µg/µL). You will need 15 µL of the mix per sample.

Sample calculation for 54 samples:

- 54 samples x 15 µL antibody mix/sample = 810 µL total antibody mix
- Always make up a little extra, so prepare a total of 900 µL.
- 900 µL x 0.02 µg/µL antibody = 18 µg antibody

- 18 µg antibody / (10 µg/µL) (concentration of anti-FLAG antibody) = 1.8 µL antibody
- Mix 1.8 µL antibody with 87 µL Zenon Alexa Fluor 488 and incubate 5 minutes at room temperature (concentration of this mix is 0.2 µg/µL → 18 µg antibody / 90 µL total volume).
- Dilute 1:10 → add 810 µL TBS containing 4% BSA for a total volume of 900 µL (final concentration of antibody is 0.02 µg/µL.

b. In a Corning 96-well round bottom plate (Corning #3788) add 10 µL of cells and 15 µL of the antibody mix. Mix with a vortexer set at a slow speed.

c. Incubate the plate at 4˚C for 20 minutes.

d. Add 175 µL of TBS.

e. Read the plate on the Guava EasyCyte.

References

Hanson MA, Brooun A, Baker KA, Jaakola VP, Roth C, Chien EY, Alexandrov A, Velasquez J, Davis L, Griffith M, Moy K, Ganser-Pornillos BK, Hua Y, Kuhn P, Ellis S, Yeager M, Stevens RC., "Profiling of membrane protein variants in a baculovirus system by coupling cell-surface detection with small-scale parallel expression.", Protein Expr Purif. (2007) ;**56**(1):85-92.