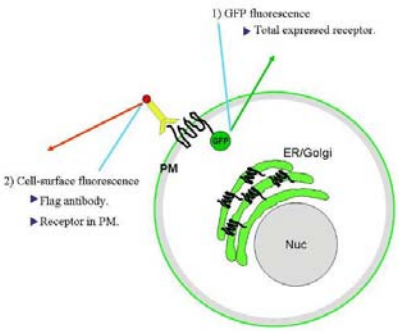
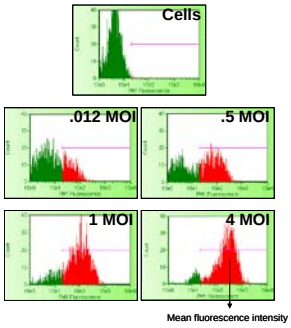
Guava Flow Cytometer

Manufacturer: Guava Technologies
Model: EasyCyte Plus System
Primary Use: This 96-well cytometer is used to monitor cell density and health, and evaluate membrane protein expression

Description:

A critical component of the JCIMPT integral membrane protein production process is the Guava flow cytometer which is routinely used for measuring cell surface expression using a protocol developed in collaboration with Guava technologies. In addition to measuring cell-surface expression using fluorescence tagged anti-FLAG antibody, we have also measured total expression by introducing a GFP tag on the C-terminal end. As shown in figure below, the protocol consists of observing the buildup of GFP fluorescence indicating the expression of GFP-tagged proteins as well as the buildup of fluorescence tagged antibody which had been designed to bind to the protein under study. Thus, in addition to monitoring protein expression, the use of the double tag allows us to distinguish between proteins inserted into the periplasmic membrane, and hence available for antibody binding, and those trapped in the endoplasmic reticulum (ER) which we assume represents unfolded proteins .





Monitoring surface expressed proteins – Green Flourescence Protein flourescence measures total proteins expressed while Cell-surface flourescence (red signal) measures that inserted in the membrane and available on the outside surface.

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.

Roth CB, Hanson MA, Stevens RC.," Stabilization of the human beta2-adrenergic receptor TM4-TM3-TM5 helix interface by mutagenesis of Glu122(3.41), a critical residue in GPCR structure", J Mol Biol. (2008)**376**(5):1305-19