Assay Protocol

FluoHitSeeker LHCGR

Catalog #: P30177-F

1. Introduction

FluoHitSeeker reporter assays are used widely to investigate cellular signaling pathways and as high-throughput screening tools for drug discovery. The FluoHitSeeker HEK293 cell lines are a clonal derivative of Human Embryonic Kidney 293 (HEK 293) cells. These cells contain a turboGFP gene (tGFP) under the control of a minimal promoter with cAMP Response Elements (CREs). CRE is the DNA-binding sequence for the transcription factor CRE binding protein (CREB), which is responsible for the regulation of a variety of biological functions including cell proliferation, circadian rhythms and memory. The Luteinizing Hormone - Choriogonadotropin Receptor or LH/CG receptor (LHCGR) FluoHiTSeeker_HEK293 cell line is designed for High throughput screening (HTS) analysis of receptor response that results in modulation of CREB activities. Elevation of the intracellular cAMP level activates cAMP response element binding protein (CREB) to bind CRE and induces the expression of the fluorescent protein turboGFP.

2. Product Components and Storage Conditions

Product: LHCGR FluoHiTSeeker cat.nº. P30117-F

Size: 2 vials $3x10^6$cells in Freezing Media.

3. Biological Activity:

This cell line is validated for cellular response to stimulation by Follicle-stimulating hormone.

Warranty

Cell Line Stability

Cells may undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

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Mycoplasma testing

The cell line has been screened using the PCR-basedVenor™GeM Mycoplasma Detection kit (Minerva) to confirm the absence of Mycoplasma species.

Storage

Immediately upon receipt, store in liquid nitrogen.

4. Materials to Be Supplied by the User

Recommended Reagents

DMEM, high glucose (Sigma-Aldrich D6429)

Fetal bovine serum (FBS)

DPBS with calcium and magnesium (Sigma Aldrich D8662)

Opti-MEM (Life technologies 31985-070)

5. Supplies and Equipment

96-well assay plate

Tissue culture flasks

Class II biological safety cabinet

Hemocytometer

Incubator humidified 37°C, 5% CO_2

Inverted microscope

Fluorimeter

6. Experimental protocol

1. Harvest LHCGR FluoHiTSeeker-HEK293 cells from culture in growth medium and seed cells at a density of ~50,000 cells per well in 200 µl of culture medium into a white clear-bottom 96-well microplate.

2. Incubate cells at 37°C in a CO_2 incubator overnight (~ 16-18 hours).

3. Remove the media and add three fold serial dilution of Luteinizing Hormone/Choriogonadotropin (hCG) in Opti-MEM to stimulated wells. Add Opti-MEM including the vehicle of the compounds to unstimulated control wells. Set up each treatment for at least triplicate.

4. Incubate the plate at 37°C in a CO2 incubator for 16-24 hours.

5. Remove the assay medium and replace it by 100µl of DPBS with calcium and magnesium. Read the plate using the appropriate filter for the tGFP protein fluorescent signal (excitation/ emission max = 482/ 502 nm).

6. Data Analysis: Subtract average background fluorescence (hCG-free control wells) from fluorescence reading of all wells.