

APPENDIX

CELL LINE DESIGNATION

Luteinizing hormone/choriogonadotropin receptor cell line
(CB-80200-282)

ORIGIN (PARENTAL CELL)

HEK 293-CNG cell

GENE INTRODUCED

Genbank LocusID 3973

RECEPTOR INTRODUCED:

Human luteinizing hormone/choriogonadotropin receptor
(NCBI protein database NP_000224.2)

USAGE

- cAMP assay for Gs-coupled human luteinizing hormone/choriogonadotropin receptor.

QUALITY CONTROL

- This cell line has been tested negative for *Mycoplasma sp.*
- This cell line has been tested positive for luteinizing hormone/choriogonadotropin receptor specific response.
- Surviving rate: More than 2.5 million/vial on the second day after thawing.
- The receptor specific activity is stable for 10 weeks continuous passage.

CELL CULTURE CONDITION

- Growth medium: 90% DMEM, 10% FBS, 250 µg/ml G418 and 1 µg/ml puromycin
- Freezing medium: 10% DMSO, 90% FBS

DATA EXAMPLE

A

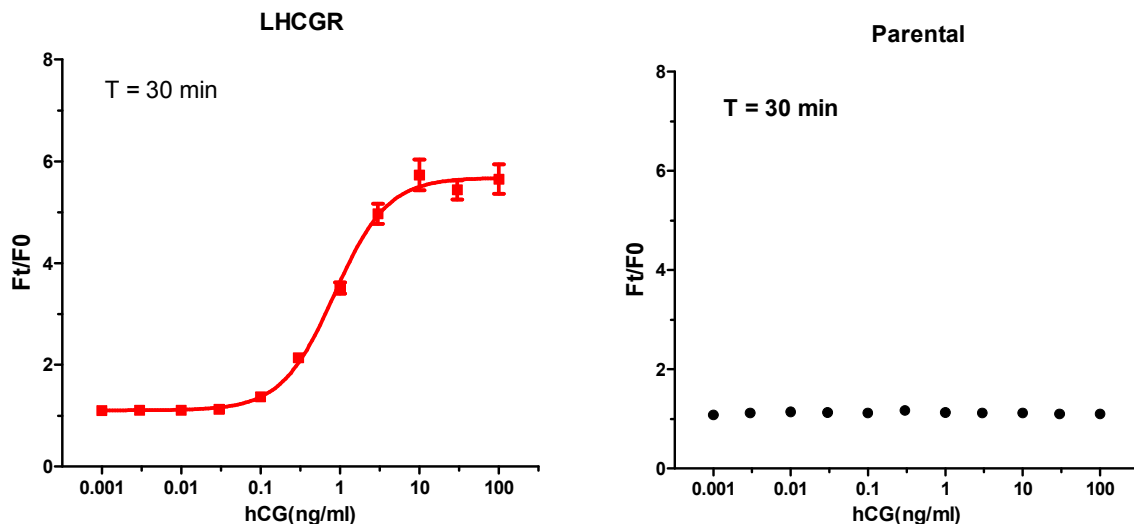


Figure 1. Response of ACTOne LHCGR cell line to hCG.

ACTOne LHCGR cells were plated overnight in 20 µl culture medium on a BD Biocoat 384 well plate. The next day, cells were dye-loaded with 20 µl/well of 1X Dye-loading solution (ACTOne Membrane Potential Assay Kit). After 2 hours of incubation at room temperature, two readings were obtained prior to and 30 min after the addition of hCG. Ratios of the two readings (F/F0) are plotted in the figure.

- Dose response curve of hCG in ACTOne LHCGR cell line. EC50 = 855 pg/ml in the presence of 25 µM of PDE inhibitor Ro20-1724.
- The parental cells do not respond to hCG