eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ LH Glycoprotein Hormone Receptor Frozen Cells

CATALOG NUMBER: HTS233LRTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

ChemiBrite cells co-express a GPCR along with a novel variant of clytin, a calcium-activated photoprotein, to enable sensitive luminescent detection of ligand-induced calcium flux. The ChemiBrite version of clytin contains a mutation that increases its affinity for calcium to a level that permits detection of cytosolic calcium in many cells with greater sensitivity than other mitochondrially expressed photoproteins. Luminescent calcium assays offer several advantages over fluorescent calcium assays including; lower substrate cost, increased sensitivity, and lack of interference from fluorescent compounds.

Luteinizing Hormone (LH), also known as lutropin, is produced by the pituitary gland and is critical for fertility and reproduction. In women, LH modulates ovulation. In men, LH stimulates testosterone production. Several naturally occurring mutations in the LH receptor gene have been associated with human reproductive disorders (Themmen *et al.*). Together with TSH, hCG and FSH, LH is a member of the glycoprotein hormone family. Each member shares a common α subunit, whereas the β subunit confers functional specificity. Upon activation, LH signals primarily through the Gαqs/adenylyl cyclase/cAMP/PKA pathway as has been shown in native granulosa cells. Cloned human LH receptor-expressing ChemiBrite cells were made by recombinant transfection of HEK293 cells with ChemiBrite clytin, the LH receptor and a promiscuous G protein to couple the receptor to the calcium signaling pathway. The cells have been cryopreserved at an optimal time post-transfection. Upon thaw, recovery, and loading, the cells are ready for luminescent, fluorescent and cAMP accumulation analysis of agonists, antagonists and modulators at the LH receptor.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

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GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

APPLICATIONS

Calcium Flux Assays, Luminescent Mode and Fluorescent Mode; cAMP accumulation

APPLICATION DATA

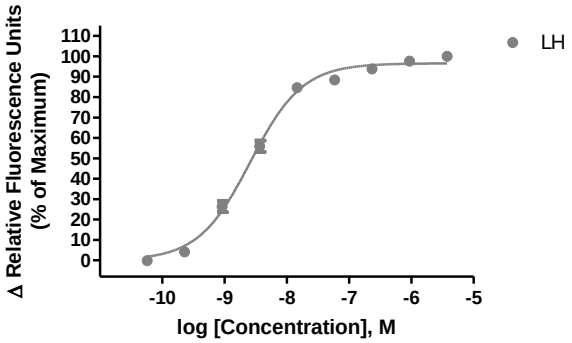

Figure 1. Representative data for activation of LH receptor. Calcium flux in LH–expressing HEK cell line induced by hCG. LH–expressing HEK cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}®. Maximal fluorescence signal obtained in this experiment was 10,000 RLU (Relative Light Units).

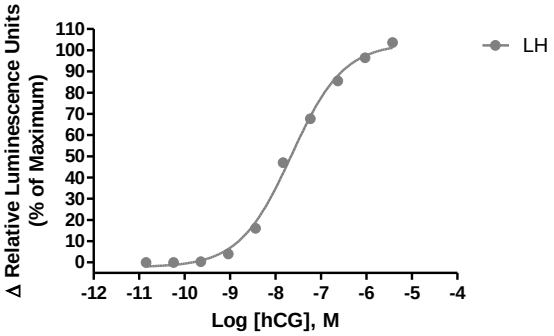

Figure 2. Representative data for activation of LH receptor stably expressed in HEK293 cells induced by human Chorionic Gonadotropin (hCG) using a luminescent calcium flux assay. LH–expressing HEK293 cells were loaded with 10 µM coelenterazine for 2h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format. Luminescence signal obtained in this experiment was 40,000 RLU (Relative Light Units) as measured by AUC (area under curve) for 80s post agonist addition using the provided protocol.

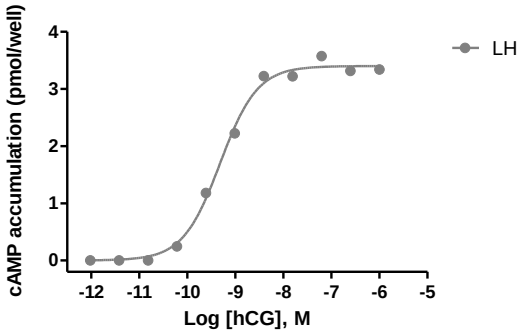

Figure 3. Representative data for activation of LH receptor stably expressed in HEK293 cells induced by hCG using a cAMP accumulation assay. LH–expressing HEK293 cells were seeded into a 96-well plate, and the following day the cells were treated with hCG for 15 minutes in the presence of 100 µM IBMX to determine receptor-mediated cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy.

Table 1. Comparison of EC_{50} values of LH-expressing HEK293 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
hCG	Calcium Flux - Fluorescence	2.6	Eurofins Internal Data
hCG	Calcium Flux - Luminescence	22	Eurofins Internal Data
hCG	cAMP accumulation	0.5	Eurofins Internal Data
LH	cAMP accumulation	0.4	Koppen *et al.* (2008)

ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.
10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-

495 nm (FLIPR^{TETRA}) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR^{TETRA}) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.

12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).

13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

HEK293

EXONGENOUS GENE EXPRESSION

Human LHCGR cDNA (Accession Number: BC156303; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein and promiscuous G protein expressed in a bicistronic vector.

CODING SEQUENCE

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ATG AAG CAG CGG TTC TCG GCG CTG CAG CTG CTG AAG CTG CTG CTG CTG CTG CAG CCG CCG CTG CCA
 M   K   Q   R   F   S   A   L   Q   L   L   K   L   L   L   L   L   Q   P   P   L   P

CGA GCG CTG CGC GAG GCG CTC TGC CCT GAG CCC TGC AAC TGC GTG CCC GAC GGC GCC CTG CGC TGC
 R   A   L   R   E   A   L   C   P   E   P   C   N   C   V   P   D   G   A   L   R   C

CCC GGC CCC ACG GCC GGT CTC ACT CGA CTA TCA CTT GCC TAC CTC CCT GTC AAA GTG ATC CCA TCT
 P   G   P   T   A   G   L   T   R   L   S   L   A   Y   L   P   V   K   V   I   P   S

CAA GCT TTC AGA GGA CTT AAT GAG GTC ATA AAA ATT GAA ATC TCT CAG ATT GAT TCC CTG GAA AGG
 Q   A   F   R   G   L   N   E   V   I   K   I   E   I   S   Q   I   D   S   L   E   R

ATA GAA GCT AAT GCC TTT GAC AAC CTC CTC AAT TTG TCT GAA ATA CTG ATC CAG AAC ACC AAA AAT
 I   E   A   N   A   F   D   N   L   L   N   L   S   E   I   L   I   Q   N   T   K   N

CTG AGA TAC ATT GAG CCC GGA GCA TTT ATA AAT CTT CCC CGA TTA AAA TAC TTG AGC ATC TGT AAC
 L   R   Y   I   E   P   G   A   F   I   N   L   P   R   L   K   Y   L   S   I   C   N

ACA GGC ATC AGA AAG TTT CCA GAT GTT ACG AAG GTC TTC TCC TCT GAA TCA AAT TTC ATT CTG GAA
 T   G   I   R   K   F   P   D   V   T   K   V   F   S   S   E   S   N   F   I   L   E

ATT TGT GAT AAC TTA CAC ATA ACC ACC ATA CCA GGA AAT GCT TTT CAA GGG ATG AAT AAT GAA TCT
 I   C   D   N   L   H   I   T   T   I   P   G   N   A   F   Q   G   M   N   N   E   S

GTA ACA CTC AAA CTA TAT GGA AAT GGA TTT GAA GAA GTA CAA AGT CAT GCA TTC AAT GGG ACG ACA
 V   T   L   K   L   Y   G   N   G   F   E   E   V   Q   S   H   A   F   N   G   T   T

CTG ACT TCA CTG GAG CTA AAG GAA AAC GTA CAT CTG GAG AAG ATG CAC AAT GGA GCC TTC CGT GGG
 L   T   S   L   E   L   K   E   N   V   H   L   E   K   M   H   N   G   A   F   R   G

GCC ACA GGG CCG AAA ACC TTG GAT ATT TCT TCC ACC AAA TTG CAG GCC CTG CCG AGC TAT GGC CTA
 A   T   G   P   K   T   L   D   I   S   S   T   K   L   Q   A   L   P   S   Y   G   L

GAG TCC ATT CAG AGG CTA ATT GCC ACG TCA TCC TAT TCT CTA AAA AAA TTG CCA TCA AGA GAA ACA
 E   S   I   Q   R   L   I   A   T   S   S   Y   S   L   K   K   L   P   S   R   E   T

TTT GTC AAT CTC CTG GAG GCC ACG TTG ACT TAC CCC AGC CAC TGC TGT GCT TTT AGA AAC TTG CCA
 F   V   N   L   L   E   A   T   L   T   Y   P   S   H   C   C   A   F   R   N   L   P

ACA AAA GAA CAG AAT TTT TCA CAT TCC ATT TCT GAA AAC TTT TCC AAA CAA TGT GAA AGC ACA GTA
 T   K   E   Q   N   F   S   H   S   I   S   E   N   F   S   K   Q   C   E   S   T   V

AGG AAA GTG AAT AAC AAA ACA CTT TAT TCT TCC ATG CTT GCT GAG AGT GAA CTG AGT GGC TGG GAC
 R   K   V   N   N   K   T   L   Y   S   S   M   L   A   E   S   E   L   S   G   W   D

TAT GAA TAT GGT TTC TGC TTA CCC AAG ACA CCC CGA TGT GCT CCT GAA CCA GAT GCT TTT AAT CCC
 Y   E   Y   G   F   C   L   P   K   T   P   R   C   A   P   E   P   D   A   F   N   P

TGT GAA GAT ATT ATG GGC TAT GAC TTC CTT AGG GTC CTG ATT TGG CTG ATT AAT ATT CTA GCC ATC
 C   E   D   I   M   G   Y   D   F   L   R   V   L   I   W   L   I   N   I   L   A   I

ATG GGA AAC ATG ACT GTT CTT TTT GTT CTC CTG ACA AGT CGT TAC AAA CTT ACA GTG CCT CGT TTT
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 L   M   C   N   L   S   F   A   D   F   C   M   G   L   Y   L   L   L   I   A   S   V

GAT TCC CAA ACC AAG GGC CAG TAC TAT AAC CAT GCC ATA GAC TGG CAG ACA GGG AGT GGG TGC AGC
 D   S   Q   T   K   G   Q   Y   Y   N   H   A   I   D   W   Q   T   G   S   G   C   S

ACT GCT GGC TTT TTC ACT GTA TTC GCA AGT GAA CTT TCT GTC TAC ACC CTC ACC GTC ATC ACT CTA
 T   A   G   F   F   T   V   F   A   S   E   L   S   V   Y   T   L   T   V   I   T   L

GAA AGA TGG CAC ACC ATC ACC TAT GCT ATT CAC CTG GAC CAA AAG CTG CGA TTA AGA CAT GCC ATT
 E   R   W   H   T   I   T   Y   A   I   H   L   D   Q   K   L   R   L   R   H   A   I

CTG ATT ATG CTT GGA GGA TGG CTC TTT TCT TCT CTA ATT GCT ATG TTG CCC CTT GTC GGT GTC AGC
 L   I   M   L   G   G   W   L   F   S   S   L   I   A   M   L   P   L   V   G   V   S

AAT TAC ATG AAG GTC AGT ATT TGC TTC CCC ATG GAT GTG GAA ACC ACT CTC TCA CAA GTC TAT ATA
 N   Y   M   K   V   S   I   C   F   P   M   D   V   E   T   T   L   S   Q   V   Y   I

TTA ACC ATC CTG ATT CTC AAT GTG GTG GCC TTC TTC ATA ATT TGT GCT TGC TAC ATT AAA ATT TAT
 L   T   I   L   I   L   N   V   V   A   F   F   I   I   C   A   C   Y   I   K   I   Y
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TTT GCA GTT CGA AAC CCA GAA TTA ATG GCT ACC AAT AAA GAT ACA AAG ATT GCT AAG AAA ATG GCA
 F   A   V   R   N   P   E   L   M   A   T   N   K   D   T   K   I   A   K   K   M   A

ATC CTC ATC TTC ACC GAT TTC ACC TGC ATG GCA CCT ATC TCT TTT TTT GCC ATC TCA GCT GCC TTC
 I   L   I   F   T   D   F   T   C   M   A   P   I   S   F   F   A   I   S   A   A   F

AAA GTA CCT CTT ATC ACA GTA ACC AAC TCT AAA GTT TTA CTG GTT CTT TTT TAT CCC ATC AAT TCT
 K   V   P   L   I   T   V   T   N   S   K   V   L   L   V   L   F   Y   P   I   N   S

TGT GCC AAT CCA TTT CTG TAT GCA ATA TTC ACT AAG ACA TTC CAA AGA GAT TTC TTT CTT TTG CTG
 C   A   N   P   F   L   Y   A   I   F   T   K   T   F   Q   R   D   F   F   L   L   L

AGC AAA TTT GGC TGC TGT AAA CGT CGG GCT GAA CTT TAT AGA AGG AAA GAT TTT TCA GCT TAC ACC
 S   K   F   G   C   C   K   R   R   A   E   L   Y   R   R   K   D   F   S   A   Y   T

TCC AAC TGC AAA AAT GGC TTC ACT GGA TCA AAT AAG CCT TCT CAA TCC ACC TTG AAG TTG TCC ACA
 S   N   C   K   N   G   F   T   G   S   N   K   P   S   Q   S   T   L   K   L   S   T

TTG CAC TGT CAA GGT ACA GCT CTC CTA GAC AAG ACT CGC TAC ACA GAG TGT TAA
 L   H   C   Q   G   T   A   L   L   D   K   T   R   Y   T   E   C  stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTS178LRTA	ChemiBrite™ FSH Glycoprotein Hormone Receptor Frozen Cells

REFERENCES

1. Koppen *et al.* (2008) A signaling-selective, nanomolar potent allosteric low molecular weight agonist for the human luteinizing hormone receptor. *Arch. Pharm.*(378)5: 503-514
2. Jia X. *et al.* (1991) Expression of Human Luteinizing Hormone Receptor: Interaction with LH and Chorionic Gonadotropin from Human but not Equine, Rat and Ovine Species. *Mol. Endocrinology* 5 (6)
3. Themmen A. *et al.* (2000) Mutations of Gonadotropins and Gonadotropin Receptors: Elucidating the Physiology and Pathophysiology of Pituitary-Gonadal Function. *Endocrine Review* (21)5: 551-583

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