eurofins | Discovery Services

PRODUCT DATASHEET

ChemiBrite™ LH Glycoprotein Hormone Receptor Stable Cell Line

CATALOG NUMBER: HTS233L

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiBrite cells express 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 photoproteins targeted to the mitochondria. Luminescent calcium assays offer several advantages over fluorescent calcium assays including 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 stable transfection of HEK293 cells with ChemiBrite clytin, the LH receptor and a promiscuous G protein to couple the receptor to the calcium signaling pathway. These stability-tested cells are ready for luminescent analysis of agonists, antagonists and modulators at the LH receptor.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

WARNINGS

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

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

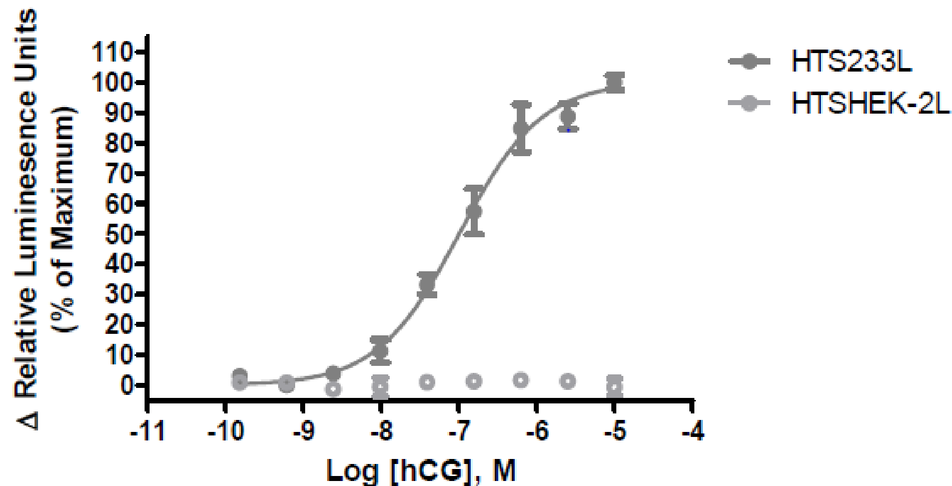

Figure 1. Representative data for activation of LH receptor stably expressed in HEK293 cells induced by human Chorionic Gonadotropin (hCG) using a luminescent calcium flux assay. LH–expressing HEK293 cells were loaded with 10 μM coelenterazine for 3 h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPRTETRA® with ICCD camera in 96-well format with a final concentration of 0.5% DMSO. Luminescence signal obtained in this experiment was 19,000 RLU (Relative Light Units) as measured by AUC (area under curve) for 80 s post agonist addition using the provided protocol. Similarly parental cells (catalog #: HTSHEK-2L) were tested to determine the specificity of the resulting signal.

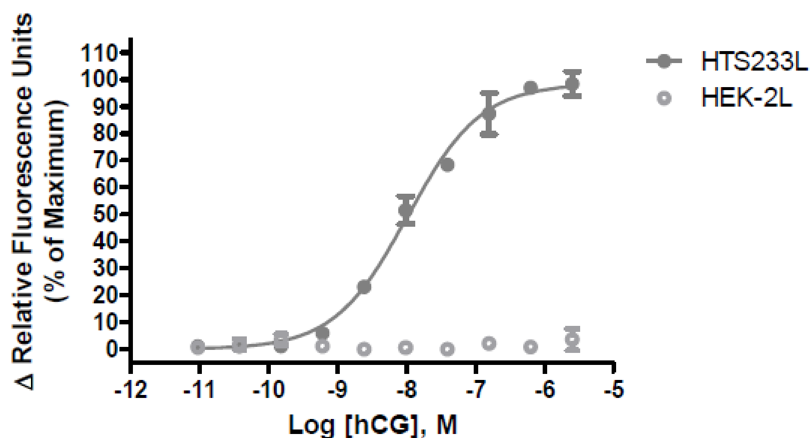

Figure 2. Representative data for activation of LH receptor stably expressed in HEK293 cells induced by human hCG using a fluorescent calcium flux assay. LH-expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a calcium dye. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPRTETRA® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 8,000 RLU. Similarly parental cells (catalog #: HTSHEK-2L) were tested to determine the specificity of the resulting signal.

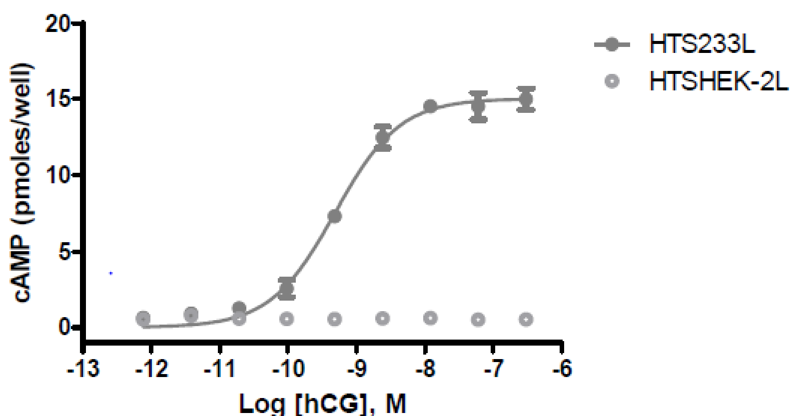

Figure 3. Representative data for activation of LH receptor stably expressed in HEK293 cells induced by hCG using a cAMP accumulation assay. LH–expressing HEK293 cells were seeded at 25,000 cells per well into a 96-well plate, and the following day the cells were treated with ligand for 10 minutes in the presence of 2 mM 3-isobutyl-1-methylxanthine (IBMX) and 0.5% DMSO to determine receptor-mediated cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Similarly parental cells (catalog #: HTSHEK-2L) were tested to determine the specificity of the resulting signal.

Table 1. EC50 values of LH-expressing HEK293 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
hCG	Calcium Flux - Luminescence	100	Eurofins Internal Data
hCG	Calcium Flux - Fluorescence	10	Eurofins Internal Data
hCG	cAMP accumulation	1.0	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC50 and signal at 1, 3 and 6 weeks of continuous culture by calcium flux luminescence. The Z' value, as defined with response to 10uM hCG is 0.7.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM/F12	-	Millipore: DF-041-B
	Fetal Bovine Serum (FBS)	10%	Gibco: 16000
	Non-Essential Amino Acids (NEAA)	1X	Millipore: TMS-001-C
Selection Medium	Basal Medium (see above)	-	
	Puromycin	1 ug/ml	EMD: 400053
	Geneticin (G418)	200 µg/ml	Invivogen: ant-gn-5
	Hygromycin	100 µg/ml	Invivogen: ant-hg-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.05% Trypsin-EDTA	-	Millipore: SM-2002-B
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Gibco: 16000
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min.* Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	3.0	24
T75	15	2.0	48
T75	15	1.5	72

ASSAY SETUP

Luminescence

Table 4. Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Luminescence
Ex/Em	None/None
Camera Gain	280,000
Gate Open	100 %
Exposure Time	0.9 sec
Read Interval	1 sec.
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
BSA (Protease Free). Prepare to 1% in H_2O, filter	EMD: 126609
hCG hormone (5000IU/mg)	ProspecBio: HOR250
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Luminescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC, $^{5e6/}{}_{5e5/ml}$ =10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, pH 7.4) and Loading buffer (Assay buffer with 10µM coelenterazine). *Note: Please prepare coelenterazine stock according to Manufacturer's Recommendations at 10mM to allow for 1:1000 dilution into Loading buffer (10uM final concentration). It is critical that coelenterazine solution is prepared at room temperature and is protected from light.*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to $FLIPR^{TETRA}$® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. Export data to analyze using the area under the curve statistic.

HOST CELL

HEK293

EXOGENOUS 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

```
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
```

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 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

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 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
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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
 M   G   N   M   T   V   L   F   V   L   L   T   S   R   Y   K   L   T   V   P   R   F

CTC ATG TGC AAT CTC TCC TTT GCA GAC TTT TGC ATG GGG CTC TAT CTG CTG CTC ATA GCC TCA GTT
 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

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
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 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
```

RELATED PRODUCTS

Product Number	Description
HTSHEK-2L	ChemiBrite™ HEK293 Parental Stable Cell Line with Gαqs
HTS178L	ChemiBrite™ FSH Glycoprotein Hormone Receptor stable cell line
HTS027C	ChemiScreen™ GNRH Hormone Receptor stable cell line

REFERENCES

1. 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
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)

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