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PRODUCT DATASHEET

ChemiBrite™ CHO Parental Stable Cell Line

CATALOG NUMBER: HTSCHO-1L

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.

CHO cell lines provide investigators with robust platforms for recombinant expression of GPCRs and Ion Channels. We have established an adapted CHO cell line with proven success in multiple expression systems on high throughput platforms. Cloned CHO Parental ChemiBrite cells are made by stable transfection of CHO cells with ChemiBrite clytin. This photoprotein stability-tested cell line is ideal for recombinant expression in luminescent analysis of agonists, antagonists and modulators at the target protein.

USE RESTRICTIONS

Please see User Agreement (Label License) 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.

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APPLICATIONS

Luminescent Calcium Flux and cAMP assays

APPLICATION DATA

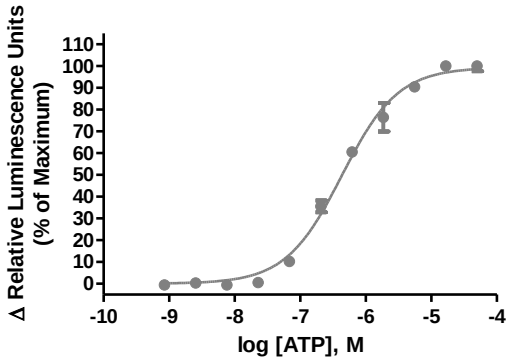

Figure 1. Representative data for luminescent calcium flux assay of CHO cells stably expressing clytin, as measured with endogenous purinergic receptor response to ATP addition. Clytin–expressing CHO cells were loaded with 10 µM coelenterazine for 3 h at room temperature and calcium flux in response to ATP was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format with a final concentration of 0.5% DMSO. Luminescence signal obtained in this experiment was 70,000 RLU (Relative Light Units) as measured by AUC (are under curve) for 80 s post agonist addition using the provided protocol.

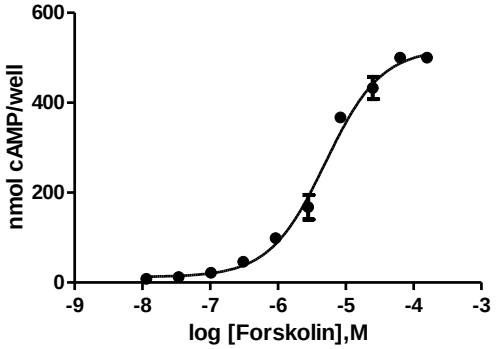

Figure 2. Representative data illustrating cAMP response in CHO cells induced by Forskolin using a cAMP accumulation assay. Clytin–expressing CHO cells were seeded at 25,000 cells per well into a 96-well plate. The following day, the cells were treated with Forskolin for 10 min in the presence of 2 mM IBMX and 0.5% DMSO. Generation of cAMP was determined using a time-resolve fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal fluorescence signal obtained in this experiment was 500 nmol cAMP/well.

Table 1. EC_{50} values of Clytin-expressing CHO cells with values obtained internally.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
ATP	Calcium Flux - Luminescence	400*	Eurofins Internal Data
Forskolin	cAMP accumulation	4900	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux luminescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM/F12 medium	-	Hyclone: SH30023
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH3007003
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH3023801
Selection Medium	Basal Medium (see above)	-	
	Puromycin	1 µg/ml	Invivogen: ant-pr-1
Dissociation	Sterile PBS	-	Hyclone: SH3025601
	0.25% Trypsin-EDTA	-	Hyclone: SH3004201
CryoMedium	Basal Medium (see above)	40%	-
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH3007003
	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 2 min, RT, until cells dislodge. *If cells do not round up, place in 37º C incubator for 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	2.0	24
T75	15	1.0	48
T75	15	0.8	72
T150	30	4.0	24
T150	30	3.0	48
T150	30	2.0	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	Millipore: TMS-003-C
BSA (Protease Free). Prepare to 1% in H_2O, filter	Merck EMD: 126609
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

CHO-K1

RELATED PRODUCTS

Product Number	Description
HTSHEK-1L	ChemiBrite™ HEK293 stable parental cell line
HTSU2OS-1L	ChemiBrite™ U2OS stable parental cell line

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