eurofins | Discovery Services

PRODUCT DATASHEET

ChemiBrite™ A_{2A} Adenosine Receptor Stable Cell Line

CATALOG NUMBER: HTS048L

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.

Extracellular adenosine mediates a multitude of biological effects, including wakefulness, antiarrythmia, bronchoconstriction and response to ischemia and oxidative stress. A family of four G protein-coupled adenosine receptors, A_1, A_{2A}, A_{2B} and A_3, is responsible for these effects. A_{2A}, which couples to G_s, is expressed in basal ganglia and immune cells. A_{2A} reduces ischemia-induced inflammation; however, A_{2A} antagonists protect from neurodegeneration during Parkinson's disease (Chen, 2003). Caffeine, the most widely used psychoactive drug, is a nonselective adenosine receptor antagonist, but its psychomotor stimulant affect is attributed to A_{2A} expressed on striatal projection neurons (Fisone et al., 2004). Cloned A_{2A} receptor-expressing ChemiBrite cells were made by stable transfection of HEK293 cells with ChemiBrite clytin and the 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 A_{2A} 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.

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APPLICATIONS

Calcium Flux Fluorescence Assay, Luminescence Assay, cAMP accumulation

APPLICATION DATA

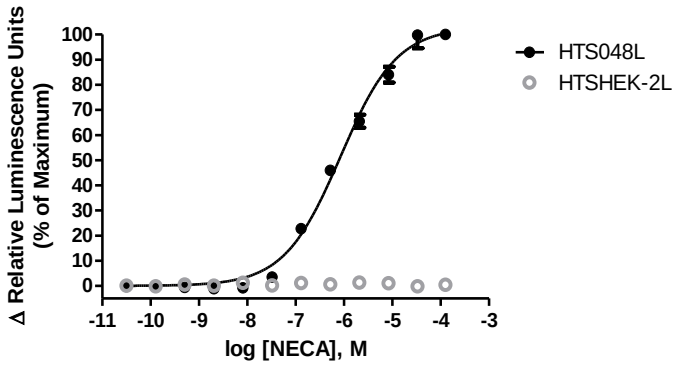

Figure 1. Representative data for activation of A_{2A} receptor stably expressed in HEK293 cells induced by NECA using a luminescent calcium flux assay. A_{2A} –expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format with a final concentration of 0.5% DMSO. Luminescence signal obtained in this experiment was 100,000 RLU (Relative Light Units) as measured by AUC (are 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.

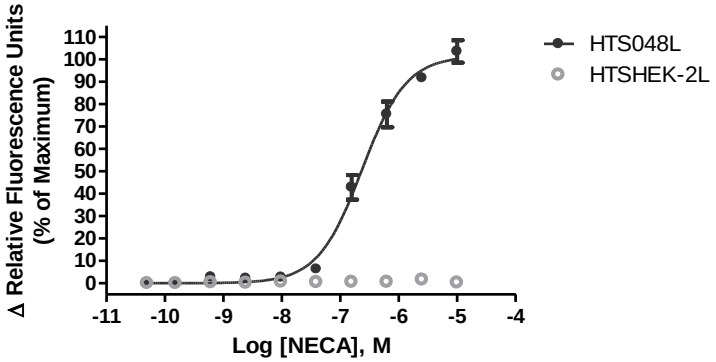

Figure 2. Representative data for activation of A_{2A} receptor stably expressed in HEK293 cells induced by NECA using a fluorescent calcium flux assay. A_{2A} –expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate. The following day, cells were loaded with a calcium dye. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 14,000 RLU. Similarly parental cells (catalog #: HTSHEK-2L) were tested to determine the specificity of the resulting signal.

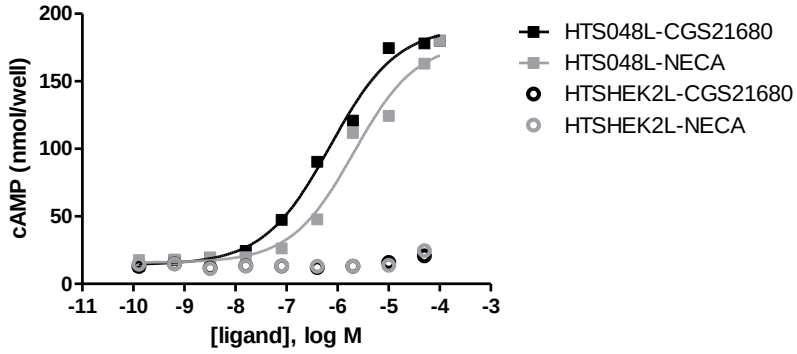

Figure 3. Representative data for activation of A_{2A} receptor stably expressed in HEK293 cells induced by Indicated Ligand using a cAMP accumulation assay. A_{2A} –expressing HEK293 cells were seeded at 25,000 cells per well into a 96-well plate, and the following day the cells were treated with ligand for 10 minutes in the presence of 100 µM 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. EC_{50} values of A_{2A}-expressing HEK293 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
CGS21680	Calcium Flux - Luminescence	2100	Eurofins Internal Data
NECA	Calcium Flux - Luminescence	2200	Eurofins Internal Data
CGS21680	Calcium Flux - Fluorescence	2000	Eurofins Internal Data
NECA	Calcium Flux - Fluorescence	2100	Eurofins Internal Data
CGS21680	cAMP accumulation-HTS048L	723	Eurofins Internal Data
NECA	cAMP accumulation-HTS048L	2000	Eurofins Internal Data
CGS21680	cAMP accumulation-Platelets	980	Cooper et al, *Br J Clin Pharm*, 1995
NECA	cAMP accumulation-Platelets	2600	Cooper et al, *Br J Clin Pharm*, 1995

* 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 fluorescence. The Z' value, as defined with response to 10µM NECA, was 0.7.

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: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Puromycin	1 ug/ml	Invivogen: ant-pr-1
	Geneticin (G418)	400 µg/ml	Invivogen: ant-gn-5
	Hygromycin	200 µg/ml	Invivogen: ant-hg-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	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	1.0	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

Fluorescence

Table 5. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 6. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8^{TM}, AM	AAT Bioquest: 21080
NECA ligand	Sigma: E2387
CGS21680 ligand	Tocris: 1063
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.

Assay Protocol – Fluorescence

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, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
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. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

HEK293

EXOGENOUS GENE EXPRESSION

Human ADORA2A cDNA (Accession Number: BC017830; 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 CCC ATC ATG GGC TCC TCG GTG TAC ATC ACG GTG GAG CTG GCC ATT GCT GTG CTG GCC
 M   P   I   M   G   S   S   V   Y   I   T   V   E   L   A   I   A   V   L   A

ATC CTG GGC AAT GTG CTG GTG TGC TGG GCC GTG TGG CTC AAC AGC AAC CTG CAG AAC GTC
 I   L   G   N   V   L   V   C   W   A   V   W   L   N   S   N   L   Q   N   V

ACC AAC TAC TTT GTG GTG TCA CTG GCG GCG GCC GAC ATC GCA GTG GGT GTG CTC GCC ATC
 T   N   Y   F   V   V   S   L   A   A   A   D   I   A   V   G   V   L   A   I

CCC TTT GCC ATC ACC ATC AGC ACC GGG TTC TGC GCT GCC TGC CAC GGC TGC CTC TTC ATT
 P   F   A   I   T   I   S   T   G   F   C   A   A   C   H   G   C   L   F   I

GCC TGC TTC GTC CTG GTC CTC ACG CAG AGC TCC ATC TTC AGT CTC CTG GCC ATC GCC ATT
 A   C   F   V   L   V   L   T   Q   S   S   I   F   S   L   L   A   I   A   I

GAC CGC TAC ATT GCC ATC CGC ATC CCG CTC CGG TAC AAT GGC TTG GTG ACC GGC ACG AGG
 D   R   Y   I   A   I   R   I   P   L   R   Y   N   G   L   V   T   G   T   R

GCT AAG GGC ATC ATT GCC ATC TGC TGG GTG CTG TCG TTT GCC ATC GGC CTG ACT CCC ATG
 A   K   G   I   I   A   I   C   W   V   L   S   F   A   I   G   L   T   P   M

CTA GGT TGG AAC AAC TGC GGT CAG CCA AAG GAG GGC AAG AAC CAC TCC CAG GGC TGC GGG
 L   G   W   N   N   C   G   Q   P   K   E   G   K   N   H   S   Q   G   C   G
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GAG GGC CAA GTG GCC TGT CTC TTT GAG GAT GTG GTC CCC ATG AAC TAC ATG GTG TAC TTC
 E   G   Q   V   A   C   L   F   E   D   V   V   P   M   N   Y   M   V   Y   F

AAC TTC TTT GCC TGT GTG CTG GTG CCC CTG CTG CTC ATG CTG GGT GTC TAT TTG CGG ATC
 N   F   F   A   C   V   L   V   P   L   L   L   M   L   G   V   Y   L   R   I

TTC CTG GCG GCG CGA CGA CAG CTG AAG CAG ATG GAG AGC CAG CCT CTG CCG GGG GAG CGG
 F   L   A   A   R   R   Q   L   K   Q   M   E   S   Q   P   L   P   G   E   R

GCA CGG TCC ACA CTG CAG AAG GAG GTC CAT GCT GCC AAG TCA CTG GCC ATC ATT GTG GGG
  A   R   S   T   L   Q   K   E   V   H   A   A   K   S   L   A   I   I   V   G

CTC TTT GCC CTC TGC TGG CTG CCC CTA CAC ATC ATC AAC TGC TTC ACT TTC TTC TGC CCC
 L   F   A   L   C   W   L   P   L   H   I   I   N   C   F   T   F   F   C   P

GAC TGC AGC CAC GCC CCT CTC TGG CTC ATG TAC CTG GCC ATC GTC CTC TCC CAC ACC AAT
  D   C   S   H   A   P   L   W   L   M   Y   L   A   I   V   L   S   H   T   N

TCG GTT GTG AAT CCC TTC ATC TAC GCC TAC CGT ATC CGC GAG TTC CGC CAG ACC TTC CGC
 S   V   V   N   P   F   I   Y   A   Y   R   I   R   E   F   R   Q   T   F   R

AAG ATC ATT CGC AGC CAC GTC CTG AGG CAG CAA GAA CCT TTC AAG GCA GCT GGC ACC AGT
 K   I   I   R   S   H   V   L   R   Q   Q   E   P   F   K   A   A   G   T   S

GCC CGG GTC TTG GCA GCT CAT GGC AGT GAC GGA GAG CAG GTC AGC CTC CGT CTC AAC GGC
 A   R   V   L   A   A   H   G   S   D   G   E   Q   V   S   L   R   L   N   G

CAC CCG CCA GGA GTG TGG GCC AAC GGC AGT GCT CCC CAC CCT GAG CGG AGG CCC AAT GGC
 H   P   P   G   V   W   A   N   G   S   A   P   H   P   E   R   R   P   N   G

TAT GCC CTG GGG CTG GTG AGT GGA GGG AGT GCC CAA GAG TCC CAG GGG AAC ACG GGC CTC
 Y   A   L   G   L   V   S   G   G   S   A   Q   E   S   Q   G   N   T   G   L

CCA GAC GTG GAG CTC CTT AGC CAT GAG CTC AAG GGA GTG TGC CCA GAG CCC CCT GGC CTA
 P   D   V   E   L   L   S   H   E   L   K   G   V   C   P   E   P   P   G   L

GAT GAC CCC CTG GCC CAG GAT GGA GCA GGA GTG TCC TGA
 D   D   P   L   A   Q   D   G   A   G   V   S  STP
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RELATED PRODUCTS

Product Number	Description
HTSHEKL	ChemiBrite™ HEK stable cell line (control cells)
HTSHEK-2L	ChemiBrite™ HEK stable cell line with Gαqs (control cells)

REFERENCES

1. Chen JF (2003) The adenosine A2A receptor as an attractive target for Parkinson's disease treatment. *Drug News Perspect*. 16: 597-604
2. Fisone G et al. (2004) Caffeine as a psychomotor stimulant: mechanism of action. *Cell Mol. Life Sci*. 61: 857-72.
3. Fredholm, BB et al. (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev*. 53: 527-552
4. Cooper et al. (1995) Adenosine Receptor-Induced cyclic AMP generation and inhibition of 5-hydroxytryptamine release in human platelets. *Br J Clin Pharm* 40:43-50

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