eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ A_{2A} Adenosine Receptor Frozen Cells

CATALOG NUMBER: HTS048LRTA

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 over night 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.

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). Eurofins Discovery Services' 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. 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 A_{2A} 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

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 Assays: Luminescent Mode and Fluorescent Mode; cAMP accumulation

APPLICATION DATA

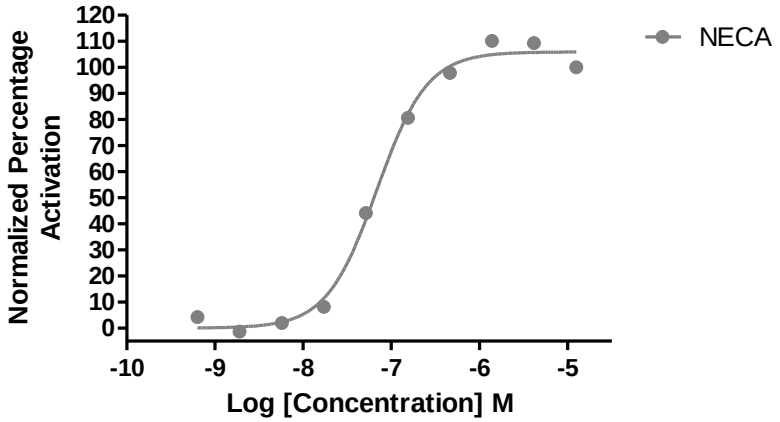

Figure 1. Representative data for activation of A_{2A} receptor. Calcium flux in A_{2A}–expressing HEK293 cell line induced by NECA. A_{2A}–expressing HEK293 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 3-fold serial dilution with each concentration performed in triplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 15,000 RLU (Relative Light Units).

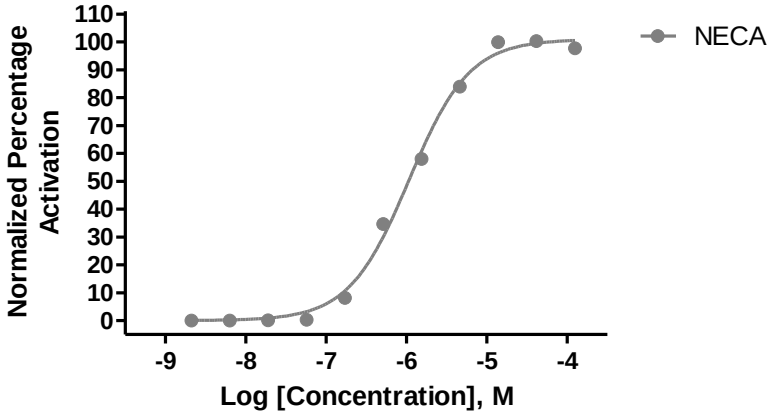

Figure 2. Representative data for activation of A_{2A} receptor expressed in HEK293 cells induced by NECA using a luminescent calcium flux assay. A_{2A}–expressing HEK293 cells were loaded with 10µM coelenterazine for 2h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format. Luminescence signal obtained in this experiment was 27,000 RLU (Relative Light Units) as measured by area-under-curve for 80s post agonist addition using the provided protocol.

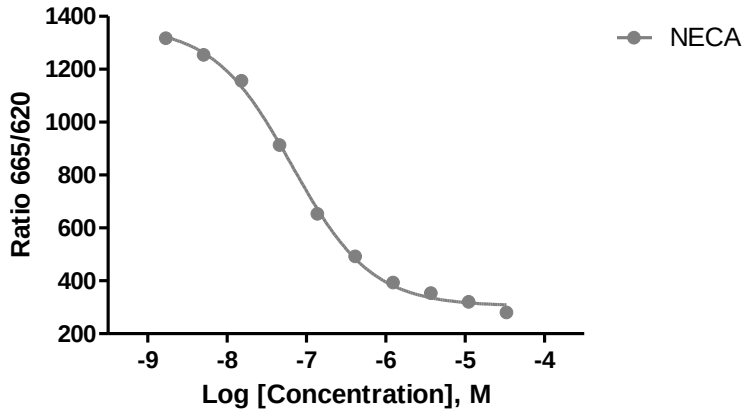

Figure 3. Representative data for activation of A_{2A} receptor stably expressed in HEK293 cells induced by NECA using a cAMP accumulation assay. A_{2A}–expressing HEK293 cells were seeded into a 96-well plate, and the following day the cells were treated with NECA for 15 minutes in the presence of 100µM 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 A_{2A}-expressing HEK293 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
NECA	Calcium Flux - Fluorescence	68	Eurofins Internal Data
NECA	Calcium Flux - Luminescence	11	Eurofins Internal Data
NECA	cAMP accumulation	66	Eurofins Internal Data

ASSAY SETUP

Luminescence

Table 2. 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 3. 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 4. 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
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 four 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

EXONGENOUS GENE EXPRESSION

Human ADAORA2A 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

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
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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)
HTS048L	ChemiBrite™ A_{2A} Receptor stable cell line

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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