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Data Sheet

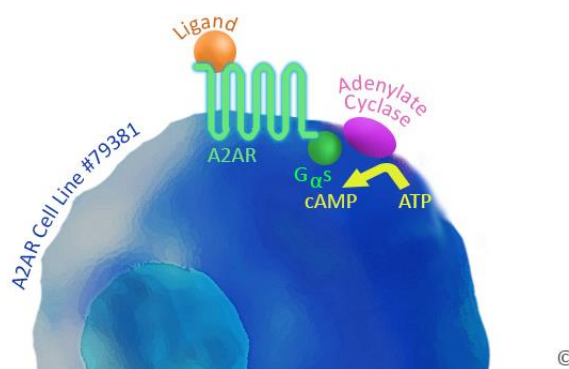
Adenosine A2A Receptor Functional Recombinant Stable Cell Line Catalog # 79381

PRODUCT DESCRIPTION:

Adenosine A2a receptor (A2aR or ADORA2A) stably expressed in HEK-293 cells. A2aR is a member of the seven transmembrane G protein-coupled receptor (GPCR) family. The activity of A2aR is mediated by G α s protein which activates adenylyl cyclase, resulting in the synthesis of intracellular cAMP. The level of cAMP correlates with the respective adenosine (agonist) level, and the cell line can be used to measure the EC50 and IC50 values of A2aR agonists or antagonists in a quantitative manner. Binding of extracellular adenosine ligand or its stable analog NECA (5'-(N-Ethylcarboxamido) adenosine) to the A2aR expressed on the surface of this cell line includes cAMP expression.

BACKGROUND:

Adenosine signaling plays an important role in inflammation and the immune response. Many cells in the tumor microenvironment express ectopic CD39 and CD73, leading to the buildup of extracellular adenosine. Engagement of adenosine with the high affinity A2a receptor (A2aR) on the surface of T cells, macrophages, NK cells, neutrophils, and dendritic cells causes downregulation of the immune response. Therefore, A2aR is a novel immune checkpoint protein, and blockade of A2aR is being actively investigated as a potential immunotherapy. Several A2aR antagonists have progressed to clinical trials for the treatment of Parkinson's disease, and preclinical studies have confirmed that blockade of A2a receptor activation has the ability to markedly enhance anti-tumor immunity. Mice treated with A2aR antagonists, such as ZM241385 (see data below) or caffeine, show significantly delayed tumor growth, and A2aR knockout mice demonstrate increased tumor rejection. Most promising, A2aR blockade can be used in synergy with the inhibition of other immune checkpoint pathways. Studies show that the combination of A2aR blockade and PD-1 inhibition is more effective than either treatment separately, and A2aR blockade increases the activity of CTLA-4 and TIM-3 inhibition in controlling the growth of CD73+ melanoma.



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APPLICATION:

- Screen for agonists or antagonists of A2aR in cell-based cAMP assays.
- Study PD-1 and CTLA-4 combination therapy.
- Screen co-inhibitor immune checkpoint molecules for cancer immunotherapy

HOST CELL:

HEK 293 cell

FORMAT:

Each vial contains ~ 2 x 10⁶ cells in 1 ml of 10% DMSO in FBS.

STORAGE:

Store in liquid nitrogen immediately upon receipt.

Thaw Medium 1 (BPS Bioscience #60187): MEM medium (Hyclone, #SH30024.01) supplemented with 10% FBS, 1% non-essential amino acids (Hyclone, #SH30238.01), 1 mM Na pyruvate (Hyclone, #SH30239.01), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01)

Growth Medium 1G (BPS Bioscience #79544): Thaw Medium 1 (BPS #60187) plus 400 µg/ml of Geneticin (Invitrogen #11811031) and 50 µg/ml of Hygromycin B (ThermoFisher, #10687010).

Cells should be grown at 37°C with 5% CO₂ using Growth Medium 1G (BPS Bioscience #79544).

RECOMMENDED CULTURE CONDITION:

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 (**no G418 or Hygromycin B**). Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 1 (**no G418 or Hygromycin B**). Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO₂ incubator. After 24 hours of culture, add an additional 3 – 4 ml of Thaw Medium 1. At first passage, switch to Growth Medium 1G (contains Thaw Medium 1, G418, and Hygromycin B). Cells should be split before they reach confluency or at ~2 x 10⁶ cells/ml.

Note: This cell line tends to grow more slowly than parental WT HEK 293 cells.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with Trypsin/EDTA, add Growth Medium 1G, and transfer to a tube. Spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add Growth Medium 1G and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

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MYCOPLASMA TESTING:

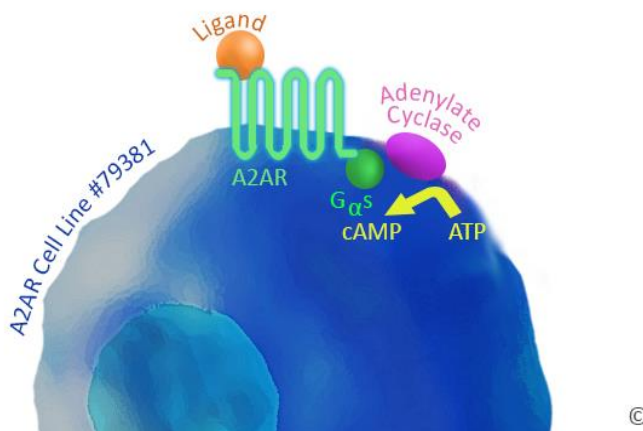
This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, #LT07-518) was used as a positive control.

REFERENCES:

1. Leone, R.D., *et al.* (2015). *Comp. Struct. Biotechnol. J.* **13**: 265-272
2. Ma, S-R., *et al.* (2017). *Molec. Cancer* **16**:99
3. Ohta, A., (2016). *Front. Immunol.* **7**:109.
4. Yang *et al.* (2017) *Purinergic Signal.* **13(2)**: 191–201
5. Varela *et al.* (2017) *Neoplasia.* 530–536 530

ASSAY PRINCIPLES:

Many cells in the tumor microenvironment express ectopic CD39 and CD73, leading to the buildup of extracellular adenosine. Engagement of adenosine with the high affinity A2a receptor (A2aR) on the surface of T cells, macrophages, NK cells, neutrophils, and dendritic cells causes downregulation of the immune response.



A2aR is a GPCR (G Protein Coupled Receptor), binding of an extracellular ligand (such as Adenosine or its analog) to its receptor A2aR alters the conformation of the associated heterotrimeric G protein, causing dissociation of the G α and G $\beta\gamma$ subunits and initiating a cascade of cellular events. The alpha subunit is categorized into one of several groups: α_s , α_i/o , α_q and $\alpha_{12/13}$. A2aR is a G α_s coupled receptor, G α_s activates adenylate cyclase, which causes cAMP increase.

cAMP can be detected using a variety of commercial cAMP assay kit, such as the cAMP-Glo™ Assay (Promega). In this assay, as the concentration of cAMP increases, cAMP binds to protein kinase A, and the regulatory subunits undergo a conformational change to release the catalytic subunits. Then the free catalytic subunits catalyze the transfer of the terminal phosphate of ATP to a protein kinase A substrate, consuming ATP in the process. The level of remaining ATP is determined using the luciferase-based Kinase-Glo® Reagent. Luminescence is inversely proportional to cAMP levels. Thus, as cAMP concentration increases, luminescence decreases.

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QUALITY ASSURANCE:

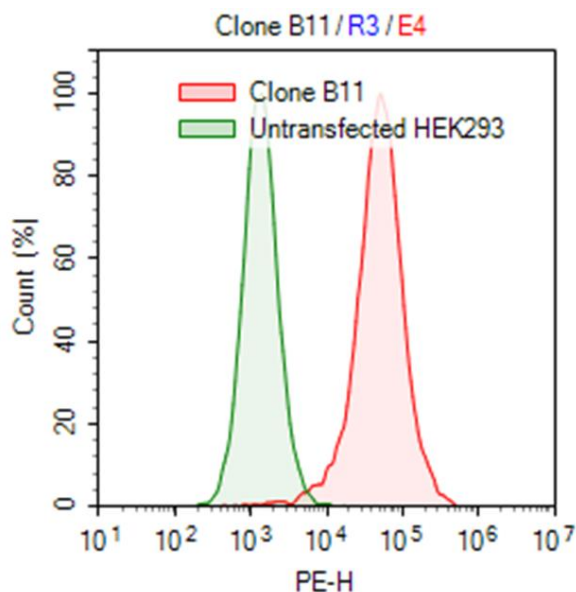


Figure 1. Expression of human A2aR on HEK293 cell surface validated by flow cytometry. Flow cytometry showed anti-human A2aR antibody (Sigma Aldrich: SAB1408884) plus PE Goat anti-mouse IgG (BioLegend, 405307) detects A2aR-positive clonal population (clone B11) (red), using wild-type HEK293 cells as a negative control (green).

MATERIALS REQUIRED BUT NOT SUPPLIED:

- Assay Medium: Thaw Medium 1 (BPS Bioscience #60187)
- Growth Medium 1G (BPS Bioscience #79544)
- Charcoal Stripped Fetal Bovine Serum (Thermo Fisher #A3382101)
- IBMX, (Sigma-Aldrich #I7018)
- Ro 20-1724, (Sigma Aldrich ##557502)
- NECA (Sigma, #E2387)
- ZM241385 (Sigma, #Z0153)
- 96-well tissue culture-treated white clear-bottom assay plate
- cAMP Glo kit (Promega, #V1501)
- Luminometer

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cAMP ASSAY PROTOCOL:

- 1) Coat poly-D-lysine-coated, white, clear-bottom 96 well plate with matrigel 30min at 37C before seeding the cells.
- 2) Seed 5000 cells/well in 200ul starvation media (MEM + 2% charcoal stripped serum), 37C o/n.
- 3) Next day, remove media carefully, wash cells 3 times with 200 μ l of warm PBS, check each well for cell density.
- 4) 1st addition: Pre-incubation with 2x antagonists (ZM241385 control) 10ul/well in induction buffer (PBS w/ 500uM IBMX + 100uM Ro 20-1724) for 15 min @ 37C.
- 5) 2nd addition: add 2x agonist (NECA at 600nM or (2x EC80)), 10ul/well for 1 hr.
- 6) Prepare cAMP standard curve for cAMP assay in a separate plate in PBS: 2 fold serial dilutions of cAMP from 4 μ M stock in a separate plate, then transfer 20 μ l/well to assay plate.
- 7) Follow cAMP-Glo™ Assay Protocols provided in the kit (Promega, #V1501) to perform cell lysis and the cAMP-Glo™ Assay (p10, 5.A.).
- 8) Read cAMP assay result on Luminometer.
- 9) Convert unknown RLU to cAMP (nM) using cAMP standard curve and Prism software.
- 10) Plot unknow cAMP (nM) from compounds against compound logM to generate EC50/IC50 curve.

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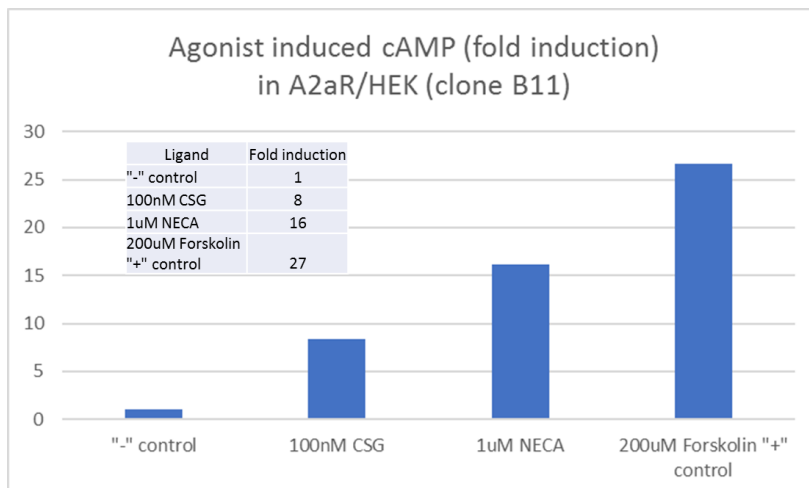


Figure 3. Agonist induced cAMP (fold induction) in A2aR/HEK293 cells
 A2aR/HEK293 cells were stimulated by various adenosine receptor agonists. The results are shown as fold induction of cAMP.

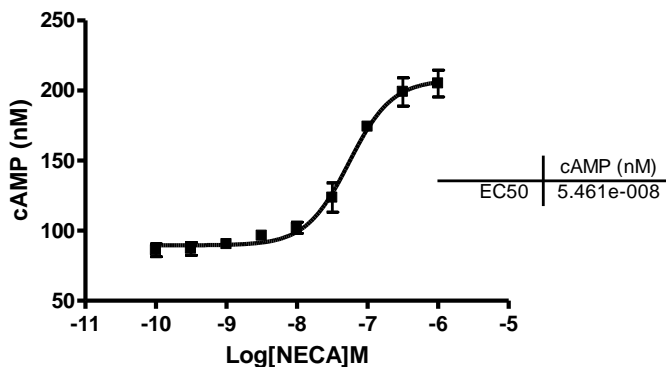


Figure 4. EC50 curve of A2aR agonist NECA using A2aR/HEK 293 functional stable cell line, measured using the cAMP Glo Assay kit (Promega)

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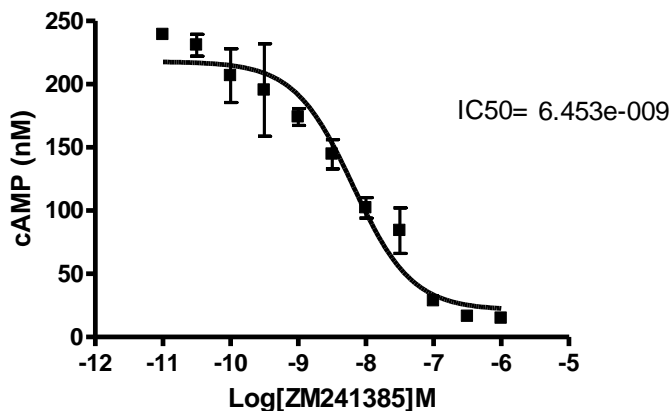


Figure 5: IC₅₀ curve of antagonist ZM241385 using A_{2a}R/HEK 293 functional stable cell line, measured using the cAMP Glo Assay kit (Promega)

VECTOR AND SEQUENCE:

Human A_{2a}R (NM_000675.5) was cloned into pcDNA3(neo)

SEQUENCE:

MPIMGSSVYITVELAIAVLAAILGNVLCWAVWLNSNLQNVNTNYFVVSLLAAADIAVGVLAIPIFAITIS
 TGFCACHGCLFIACFVLVLTQSSIFSLAIAIDRYIAIRIPLRYNGLVTGTRAKGIIAICWVLSFAIG
 LTPMLGWNNCGQPKEGKNHSQGCQVACLFEDVPMNYMVYFNFFACVLVPLLLMLGVY
 LRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIVGLFALCWLPPLHIINCFTFFCPDCS
 HAPLWLMYLAIVLSHTNSVVNPFYAYRIRREFRQTFRKIIRSHVLRQQEPFKAAGTSARVLAAHG
 SDGEQVSLRLNGHPPGVWANGSAPHPERRPNGYALGLVSGGSAQESQNGTGLPDVELLSHE
 LKGVCPPEPPGLDDPLAQDGAGVS

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CD73, His-tag	71184	50 µg
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CD73 Inhibitor Screening Assay Kit	72058	384 rxns
CD38 Inhibitor Screening Assay Kit	71275	96 rxns.
CD39 Inhibitor Screening Assay Kit	79278	96 rxns.
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