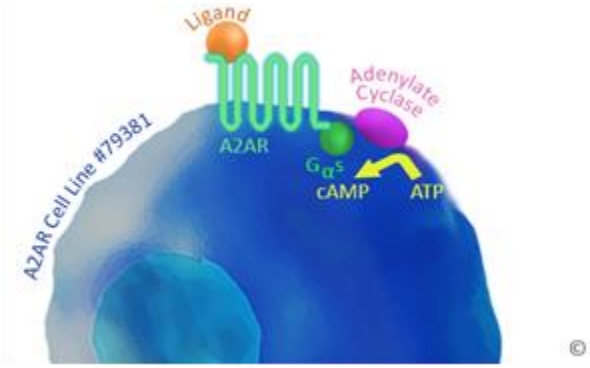
Description

Adenosine A2A receptor (A2aR or ADORA2A) stably expressed in HEK293 cells (NM_000675.5). A2aR is a member of the seven transmembrane G protein-coupled receptor (GPCR) family. The activity of A2aR is mediated by Gαs protein which activates adenylyl cyclase, resulting in the synthesis of intracellular cAMP. The level of cAMP correlates with the level of adenosine. This cell line can be used to measure the EC_{50} and IC_{50} values of A2aR agonists or antagonists.



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 Adenosine 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 A2aR activation has the ability to markedly enhance anti-tumor immunity. Mice treated with A2aR antagonists, such as ZM241385 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.

Application

- Screen or titrate agonists or antagonists of A2aR.
- Study PD-1 and CTLA-4 combination therapy.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1G	BPS Bioscience #79544

Materials Required for Cellular Assay

Name	Ordering Information
Charcoal stripped fetal bovine serum	ThermoFisher #A3382101
IBMX	Sigma-Aldrich #I7018
Ro 20-1724	Sigma Aldrich #557502
CGS-21680 hydrochloride hydrate	Sigma Aldrich #C141
ZM 241385	Sigma Aldrich #Z0153
cAMP assay kit such as	
cAMP-Gs Dynamic	PerkinElmer/Cisbio #62AM4PEB
cAMPGlo kit	Promega #V1501
96-well PDL coated white clear-bottom assay plate	Corning #354651
Plate reader to read the cAMP assay kit	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1G (BPS Bioscience #79544):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 50 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Assay Medium
MEM + 2% charcoal stripped serum.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1 **(no Hygromycin)**.
Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1 **(no Hygromycin)**.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 **(no Hygromycin)** and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1G **(contains Hygromycin)**.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1G and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1G **(contains Hygromycin)**. Seed into new culture vessels at the desired sub-cultivation ratio of [1:6 to 1:8 weekly or twice per week].

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1G and count the cells.

3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

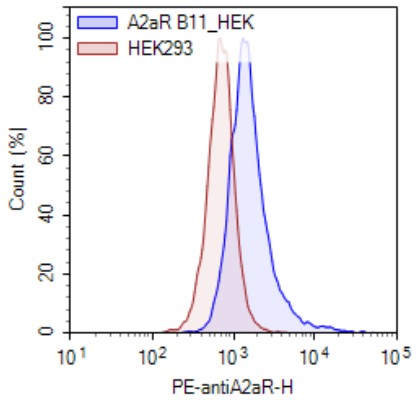


Figure 1: Expression of human A2aR on HEK293 cell surface.
Cells were incubated with a PE-labeled anti-human A2aR antibody (R&D Systems #FAB94971P-100) and analyzed by flow cytometry. Parental HEK293 cells were used as a negative control (red). An A2aR-positive clonal population (clone B11) was detected (blue).

B: Functional characterization of Adenosine A2A receptor Functional HEK293 Cell Line

Assay principles: Binding of an extracellular ligand (such as Adenosine) to A2aR alters the conformation of the associated heterotrimeric G protein, causing dissociation of the Gα and Gβγ subunits and initiating a cascade of cellular events. The alpha subunit is categorized into one of several groups: αs, αi/o, αq and α12/13. A2aR is a Gαs coupled receptor, Gαs activates adenylate cyclase, which causes an increase in cAMP. cAMP can be detected using a variety of commercial cAMP assay kits, such as the fluorescent cAMPGs assay (PerkinElmer/Cisbio) or the luminescent cAMP-Glo™ Assay (Promega)

1. Plate 10,000 cells/well in 100 µl of assay medium (MEM + 2% charcoal stripped serum) on a PDL-coated plate and incubate at 37°C in a 5% CO_2 incubator overnight.

   This cell line did not perform well when assayed in a suspension format.

2. The next day, remove the medium carefully and wash the cells twice with 200 µl of Phosphate Buffered Saline (PBS), taking care to not dislodge the cells.

a. Antagonist Assay

3. Prepare 30 µl of antagonist/well at a concentration 1.33-fold higher than the desired final concentration (the final volume will be 40 µl), using HBSS Hyclone # SH30588.02 + 500 µM IBMX + 100 µM Ro 20-1724.

4. Incubate at 37°C in a 5% CO2 incubator for 15 minutes.

5. Prepare CGS-21680 at 400 nM (4-fold more concentrated than the final desired concentration of 100 nM), using HBSS Hyclone # SH30588.02 + 500 µM IBMX + 100 µM Ro 20-1724.

6. Add 10 µl/well of CGS-21680. Other agonists may be used at their respective optimal concentration.
   Add CGS-21680 (100 nM final) to "positive control" wells that do not contain an antagonist.
   Add 10 µl/well of HBSS Hyclone # SH30588.02 + 500 µM IBMX + 100 µM Ro 20-1724 to "Negative control" wells containing no CGS-21680 and no antagonist to determine the range of the assay.

7. Incubate at 37°C in a 5% CO2 incubator for 1 hour.

8. Prepare a cAMP standard curve if desired according to the protocol from the cAMP assay manufacturer. It is recommended that each plate contains its own standard curve.

9. Perform the cAMP assay according to the manufacturer's protocol and read the plates as directed.

b. Agonist Assay

3. Prepare 30 µl of agonist/well using HBSS Hyclone # SH30588.02 + 500 µM IBMX + 100 µM Ro 20-1724.

4. Incubate at 37°C in a 5% CO2 incubator for 1 hour.

5. Prepare a cAMP standard curve if desired according to the protocol from the cAMP assay manufacturer. It is recommended that each plate contains its own standard curve.

6. Perform the cAMP assay according to the manufacturer's protocol and read the plates as directed.

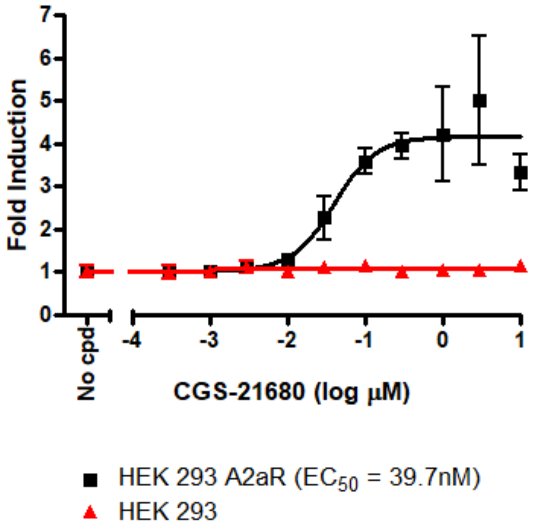


Figure 2. Agonist-induced cAMP (fold induction) in A2aR HEK293 cells.
The cells were treated with e A2aR specific agonist CGS-21680 as described in the "agonist assay" protocol above. Cells were assayed using the cAMP-Gs Dynamic (PerkinElmer/Cisbio #62AM4PEB) kit. No activation was observed in parental HEK293 cells.

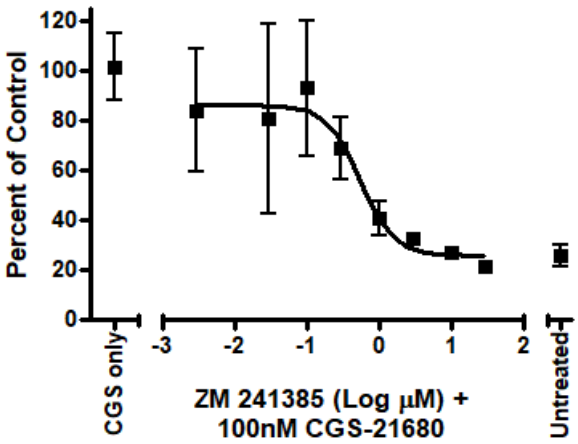


Figure 3: Antagonist dose response in A2aR HEK293 cells.
The cells were treated with increasing concentrations of A2aR antagonist ZM 241385 as described in the "antagonist assay" protocol above, in the presence of 100 nM A2aR-specific agonist CGS-21680. Cells were assayed using the cAMP-Gs Dynamic (PerkinElmer/Cisbio #62AM4PEB) kit. Results are expressed as percent of control (agonist-treated cells in the absence of antagonist, set at 100%). ZM 241385 IC_{50} = 550nM.

Sequence
Human A2aR (NM_000675.5) was cloned into pcDNA3(neo)

MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNVTNYFVVSLAAADIAVGVLAIPFAITISTGFCAACHGCLFIACFVLVLT
QSSIFSLLAIAIDRYIAIRIPLRYNGLVTGTRAKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGCGEGQVACLFEDVVPM
NYMVYFNFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVGLFALCWLPLHIINCFTFFCPD
CSHAPLWLMYLAIVLSHTNSVVNPFIYAYRIREFRQTFRKIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVW
ANGSAPHPERRPNGYALGLVSGGSAQESQGNTGLPDVELLSHE LKGVCPEPPGLDDPLAQDGAGVS

References
1. Leone RD, *et al.* (2015) *Comp. Struct. Biotechnol. J*. 13: 265-272
2. Ma S-R, *et al*. (2017) *Molec. Cancer* 16: 99
3. Ohta A, *et al.* (2016) *Front. Immunol.* 7: 109.
4. Yang et al, *et al.* (2017) *Purinergic Signal*. 13(2): 191–201
5. Mediavilla-Varela, *et al*. (2017) Neoplasia. 19(7) : 530–536

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Forskolin	27067	10 mg
CD38, His-Tag (Human), HiP™	71227	100 µg
CD73, His-tag	71184	50 µg
CD73 Inhibitor Screening Assay Kit	72055	Various Sizes
CD38 Inhibitor Screening Assay Kit	71275	96 reactions
Adenosine Deaminase (ADA), His-tag	70016	100 µg