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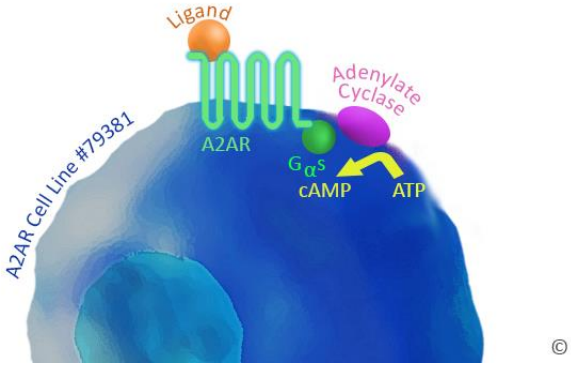
Data Sheet

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 Gαs protein which activates adenylyl cyclase, resulting in the synthesis of intracellular cAMP. The level of cAMP correlates with the respective adenosine level. This cell line can be used to measure the EC_{50} and IC_{50} values of A2aR agonists or antagonists in a quantitative manner.

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 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.
- Study PD-1 and CTLA-4 combination therapy.
- Screen co-inhibitor immune checkpoint molecules for cancer immunotherapy

HOST CELL:
HEK 293

FORMAT:
Each vial contains ~ 2 x 10^6 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 37ºC with 5% CO_2 using Growth Medium 1G (BPS Bioscience #79544).

MATERIALS REQUIRED BUT NOT SUPPLIED:

- Thaw Medium 1 (BPS Bioscience #60187)
- Growth Medium 1G (BPS Bioscience #79544)
- MEM medium (Hyclone, #SH30024.01) for preparing assay media
- Charcoal stripped fetal bovine serum (Thermo Fisher #A3382101)
- IBMX, (Sigma-Aldrich #I7018)
- Ro 20-1724, (Sigma Aldrich ##557502)
- CGS-21680 hydrochloride hydrate (Sigma, #C141)
- ZM 241385 (Sigma, #Z0153)
- 96-well PDL coated white clear-bottom assay plate (Corning #354651)
- cAMP assay kit such as:
  cAMP-Gs Dynamic (PerkinElmer/Cisbio #62AM4PEB) or cAMPGlo kit (Promega#V1501)
- Plate reader capable of reading the desired cAMP assay kit

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RECOMMENDED CULTURE CONDITION:
The user should quickly thaw the frozen cells from liquid nitrogen in a 37ºC 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 T75 flask and incubate at 37ºC in a 5% CO_2 incubator. At first passage, switch to Growth Medium 1G (contains Thaw Medium 1, Geneticin, and Hygromycin B). Cells should be split before they reach confluency. A split ratio of 1:5 or 1:10 once or twice a week is recommended.

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.

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:
A2aR is a GPCR (G Protein Coupled Receptor). 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 cAMP-Gs assay (PerkinElmer/Cisbio) or the luminescent cAMP-Glo™ Assay (Promega)

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QUALITY ASSURANCE:

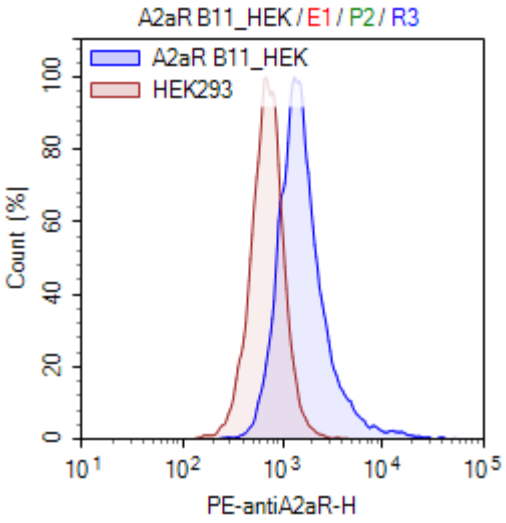

Figure 1. Expression of human A2aR on HEK293 cell surface validated by flow cytometry. Flow cytometry showing PE-labeled anti-human A2aR antibody (R&D Systems #FAB94971P-100) detects A2aR-positive clonal population (clone B11) (blue), using wild-type HEK293 cells as a negative control (red).

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cAMP ASSAY PROTOCOL:

1) Plate 10,000 cells/well in 100ul assay media (MEM + 2% charcoal stripped serum) on a PDL coated plate and incubate 37ºC 5% CO_2 overnight. This cell line did not perform well when assayed in a suspension format.

2) The next day, remove media carefully and wash the cells 2 times with 200 µls of PBS taking care to not dislodge the cells.

For antagonist assay:

3) Add 30uls of antagonist/well at 1.33x the final concentration so that the final desired dose will be achieved in 40uls (in step 5). Prepare in stimulation buffer (HBSS Hyclone # SH30588.02 or other suitable buffer) + 500uM IBMX + 100uM Ro 20-1724.

4) Incubate at 37ºC, 5% CO_2 for 15 minutes.

5) Prepare CGS-21680 at 400nM (4x the final dose) and add 10uls/well (final = 100nM). Use the same stimulation buffer as in step 3 above. Other agonists may be used at an appropriate dose. It is recommended that wells containing 100nM CGS-21680, but no antagonist in addition to wells containing no CGS-21680 and no antagonist be included to determine the range of the assay.

6) Incubate at 37ºC, 5% CO_2 for 1 hour.

7) Prepare a cAMP standard curve if desired according to the cAMP assay manufacturer's protocol and add to blank wells on the cell plate. It is recommended that each plate contain its own standard curve.

8) Optional: Remove 20uls/well from the wells with cells and discard. This step is included to reduce the amount of assay kit reagents used and thereby reduce the cost. A minimum of 30uls/well must be present prior to lysis to prevent cells from drying out.

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

For agonist assay.

3) Add 30uls of agonist/well. Prepare in stimulation buffer (HBSS or other suitable buffer) + 500uM IBMX + 100uM Ro 20-1724.

4) Incubate at 37ºC, 5% CO_2 for 1 hour and proceed w/ steps 7-9 above.

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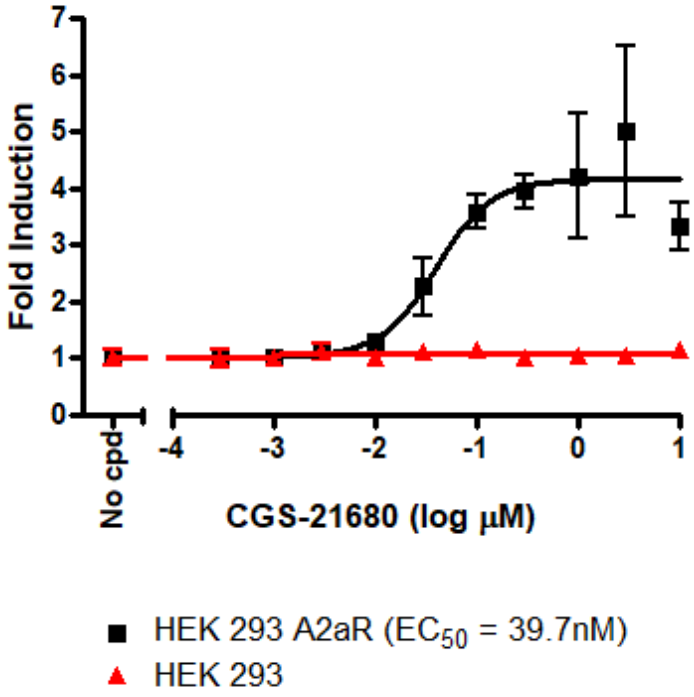

Figure 2. Agonist induced cAMP (fold induction) in A2aR/HEK293 cells
Hek 293 A2aR stable cells were treated with the A2aR specific agonist CGS-21680 as described in the "agonist assay" protocol above. No signaling was observed in parental Hek 293 cells at up to 10μM agonist. Cells were assayed using the cAMP-Gs Dynamic (PerkinElmer/Cisbio #62AM4PEB) kit.

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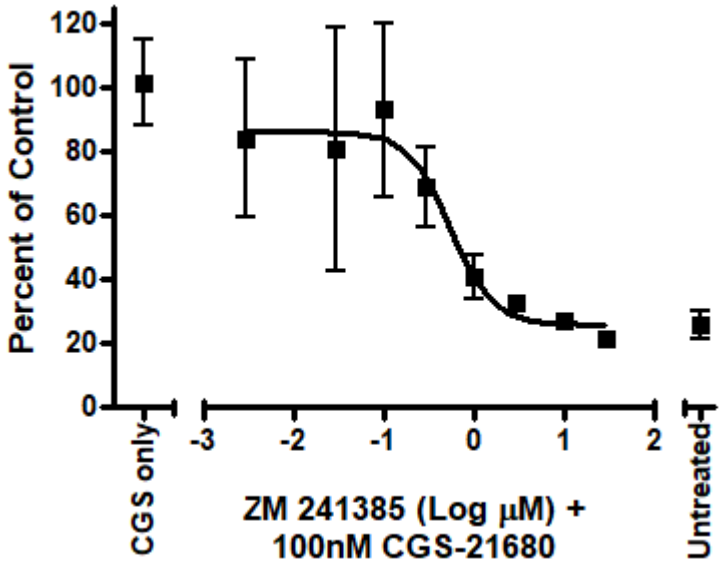

Figure 3: Antagonist dose response in A2aR Hek cells
Dose curve of the A2aR antagonist ZM 241385 run as described in the "antagonist assay" protocol above against 100nM A2aR specific agonist CGS-21680. ZM 241385 IC_{50} = 550nM. Cells were assayed using the cAMP-Gs Dynamic (PerkinElmer/Cisbio #62AM4PEB) kit.

VECTOR AND SEQUENCE:
Human A2aR (NM_000675.5) was cloned into pcDNA3(neo)

SEQUENCE:
MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNVTNYFVVSLAAADIAVGVLAIPFAITIS
TGFCAACHGCLFIACFVLVLTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGTRAKGIIAICWVLSFAIG
LTPMLGWNNCGQPKEGKNHSQGCGEGQVACLFEDVVPMNYMVYFNFFACVLVPLLLMLGVY
LRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVGLFALCWLPLHIINCFTFFCPDCS
HAPLWLMYLAIVLSHTNSVVNPFIYAYRIREFRQTFRKIIRSHVLRQQEPFKAAGTSARVLAAHG
SDGEQVSLRLNGHPPGVWANGSAPHPERRPNGYALGLVSGGSAQESQGNTGLPDVELLSHE
LKGVCPEPPGLDDPLAQDGAGVS

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Related Products	Cat. #	Size
Forskolin	27067	10 mg
CD38, His-Tag (Human), HiP™	71227	100 µg
CD39, His-tag	71284	20 µg
CD73, Avi, His-tag (Mouse)	72523	100 µg
CD73, His-tag	71184	50 µg
CD73 Inhibitor Screening Assay Kit	72055	96 rxns
CD73 Inhibitor Screening Assay Kit	72058	384 rxns
CD38 Inhibitor Screening Assay Kit	71275	96 rxns.
CD39 Inhibitor Screening Assay Kit	79278	96 rxns.
Adenosine Deaminase (ADA), His-tag	70016	100 µg