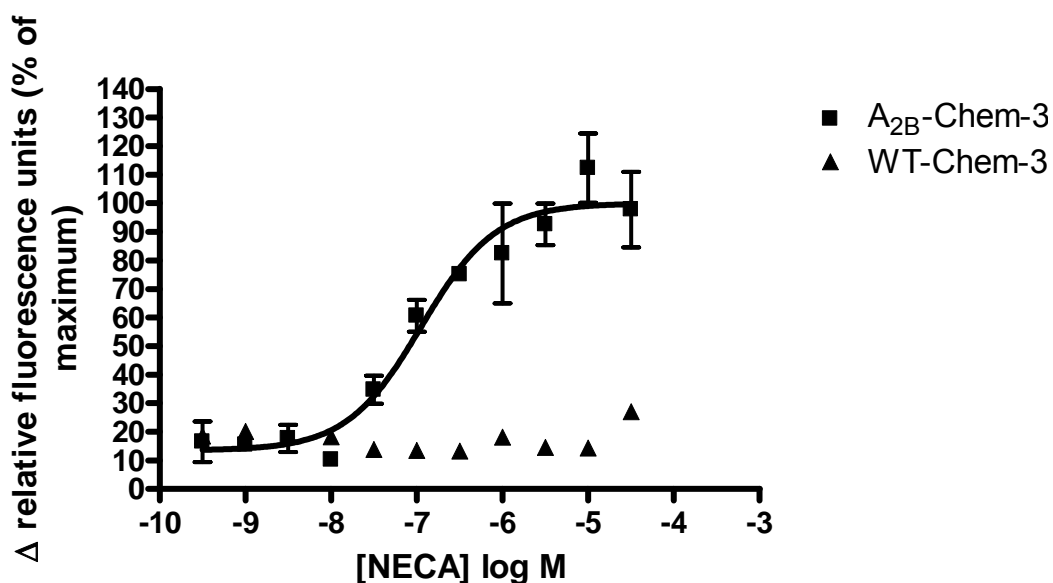


**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT A<sub>2B</sub> RECEPTOR**

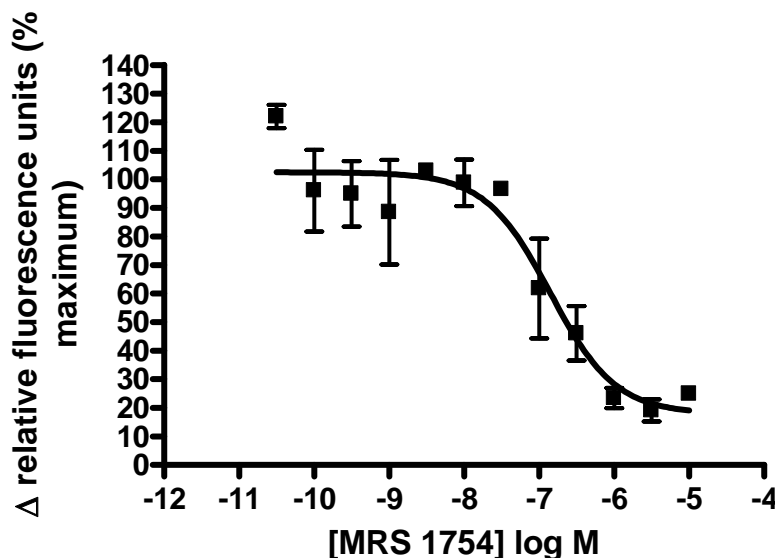
<b>CATALOG NUMBER:</b>	HTS051C	<b>QUANTITY:</b>	2 vials, 1 mL per vial
<b>LOT NUMBER:</b>		<b>CONCENTRATION:</b>	2 x 10 <sup>6</sup> cells/mL

**BACKGROUND:** Extracellular adenosine mediates a multitude of biological effects, including wakefulness, antiarrhythmia, bronchoconstriction and response to ischemia and oxidative stress. A family of four G protein-coupled adenosine receptors, A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>, is responsible for these effects. A<sub>2B</sub>, which couples to G<sub>s</sub>, is expressed in smooth muscle of the bowel, and blood vessels, and mediates relaxation of these tissues in response to adenosine (Fredholm *et al.*, 2001). Airway mast cells also express A<sub>2B</sub>, which appears to contribute to bronchoconstriction during asthma (Holgate, 2005). Chemicon's cloned human A<sub>2B</sub>-expressing cell line is made in the Chem-3 host, which supports high levels of recombinant A<sub>2B</sub> expression on the cell surface and contains high levels of the promiscuous G protein G $\alpha$ 15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at A<sub>2B</sub>.

**APPLICATIONS:** Calcium flux assay, ligand binding assays



**Figure 1.** Calcium flux in A<sub>2B</sub>-expressing Chem-3 cell line induced by 5'-(N-ethylcarboxamido)adenosine (NECA). A<sub>2B</sub>-expressing Chem-3 cells and wild-type (WT) Chem-3 cells (Chemicon catalog # HTSCHEM-3) were loaded with Fluo-4 and calcium flux in response to NECA (10<sup>-4.5</sup> to 10<sup>-10</sup> M) was determined in duplicate on a Molecular Devices Flex Station.



**Figure 2.** Assay for antagonist activity at  $A_{2B}$  by calcium flux assay.  $A_{2B}$ -expressing Chem-3 cells were loaded with Fluo-4 and washed. MRS 1754 was added to the cells at the final concentration indicated, and incubated for 10 min at 37°C. Calcium flux in response to NECA (220 nM) was determined in duplicate on a Molecular Devices FLIPR<sup>TETRA™</sup>.

SPECIFICATIONS: EC50 for calcium mobilization by NECA: ~ 110 nM  
IC50 for MRS 1754 with 2x EC50 NECA: ~141 nM

HOST CELLS: Chem-3, a suspension cell line endogenously expressing the promiscuous G protein,  $G_{\alpha 15}$ , and lacking endogenous expression of adenosine receptors.

TRANSFECTION: Full-length human ADORA2B cDNA encoding  $A_{2B}$  (Accession Number: M97759)

Cells are frozen at  $2 \times 10^6$  cells/mL in RPMI/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.

**PRESENTATION:  
STORAGE/HANDLING:**

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37°C water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol. Transfer contents of the vial to a conical tube containing 10 mL Chem-3 Growth Media. Centrifuge at 200 x g for 5 min. Remove the supernatant and resuspend the cell pellet in 20 mL Chem-3 Growth Media. Transfer to a T75 flask and place the flask in a humidified incubator at 37°C with 5%  $CO_2$ .
3. Maintain the cells in suspension between  $0.1 \times 10^6$  and  $1 \times 10^6$  cells/mL. Cells are typically passaged 1:10 every 2-3 days, or 1:20 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator. Chem-3 cells tend to proliferate rapidly, so care should be taken not to allow them to become overconfluent.

- Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count cells. Centrifuge cells at 200 x g for 5 min. Resuspend cells at  $2-5 \times 10^6$  cells/mL in Chem-3 Freezing Media. Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at  $-70^\circ\text{C}$  overnight. Store the vials in liquid nitrogen.
- Cells should be passaged at least once after thawing prior to use in calcium flux assays.

**MEDIA:**

## Chem-3 Growth Media:

RPMI-1640 containing 0.3 g/L L-glutamine, no pyruvate (Millipore SLM-140)  
 10% heat-inactivated FBS  
 100 U/ml each penicillin and streptomycin (from 100x stock, Millipore TMS-AB2-C)  
 1% Chem-3 Growth Supplement (Millipore catalog # HTSCHEM-3S)  
 G-418/Geneticin (600ug/mL)

*Note:* The Chem-3 Growth Supplement is required for growth of Chem-3 cell lines

## Chem-3 Plating Media:

RPMI-1640 containing 0.3 g/L L-glutamine, no pyruvate  
 10% heat-inactivated FBS  
 100 U/ml each penicillin and streptomycin

## Chem-3 Freezing Media:

RPMI-1640 containing 0.3 g/L L-glutamine, no pyruvate  
 20% heat-inactivated FBS  
 10% DMSO (cell culture grade)

Several calcium-sensing dyes are available that eliminate the need for washing. Any of these dyes may be used by the manufacturer's protocol, with the following recommendations for suspension cells such as Chem-3:

**RECOMMENDATIONS  
FOR CALCIUM ASSAY:**

- Cells should be passaged at least once after thawing, and should be in mid-log phase of growth at the time of the assay ( $0.8-1 \times 10^6$  cells/mL). Propagate desired number of cells;  $2 \times 10^5$  cells/well are recommended for the assay. If the cell density exceeds  $1 \times 10^6$  cells/mL or the media is becoming yellow, passage the cells again and culture for at least one day before proceeding.
- On the day of the assay, count cells and collect the desired number of cells. Centrifuge cells and resuspend at  $2 \times 10^6$  cells/ml in FLIPR assay buffer. Plate 50 $\mu$ L of cell suspension/well (96-well plate) and allow cells time to settle and equilibrate to new buffer environment at  $37^\circ\text{C}$  for at least one hour.
- Prepare a 2x dye loading solution. After the cell incubation (step 2) is complete, add 50 $\mu$ L/well of the 2x dye loading solution for a final volume of 100 $\mu$ L. Incubate the cells at  $37^\circ\text{C}$  in the dark for 30 – 60 minutes.
- For FLIPR ligand addition, set pipet tip height at 125  $\mu$ L and dispense rate of 25  $\mu$ L/sec.

**REFERENCES:**

Fredholm, BB *et al.* (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* 53: 527-552.

Holgate ST (2005) The Quintiles Prize Lecture 2004. The identification of the adenosine  $A_{2B}$  receptor as a novel therapeutic target in asthma. *Br. J. Pharmacol.* 145: 1009-15.

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HUMAN RECOMBINANT A<sub>2B</sub> RECEPTOR**

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