

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT A₃ RECEPTOR****CATALOG NUMBER:** HTS052C **QUANTITY:** 2 vials, 1 mL per vial**LOT NUMBER:** **CONCENTRATION:** 2 x 10⁶ 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 GPCR adenosine receptors, A₁, A_{2A}, A_{2B} and A₃, is responsible for these effects (Fredholm *et al.*, 2001). A₃, which couples to G_{i/o}, is expressed in mast cells along with A_{2B}. Mice lacking A₃ display reduced mast cell degranulation and bronchoconstriction in response to adenosine (Tilley *et al.*, 2003; Zhong *et al.*, 2003). Chemicon's cloned human A₃-expressing cell line is made in the Chem-3 host, which supports high levels of recombinant A₃ expression on the cell surface and contains high levels of the promiscuous G protein G α 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 A₃.

APPLICATIONS: Calcium flux assay, ligand binding assays

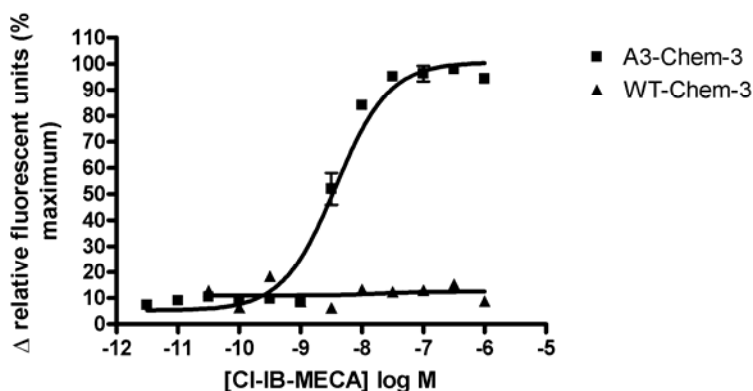


Figure 1. Calcium flux in A₃-expressing Chem-3 cell line induced CI-IB-MECA. A₃-expressing Chem-3 cells and wild-type (WT) Chem-3 cells (Chemicon catalog # HTSCHEM-3) were loaded with Fluo-4 and calcium flux in response to CI-IB-MECA (10⁻⁶ to 10^{-11.5} M) was determined in triplicate on a Molecular Devices FLIPR^{TETRA™}.

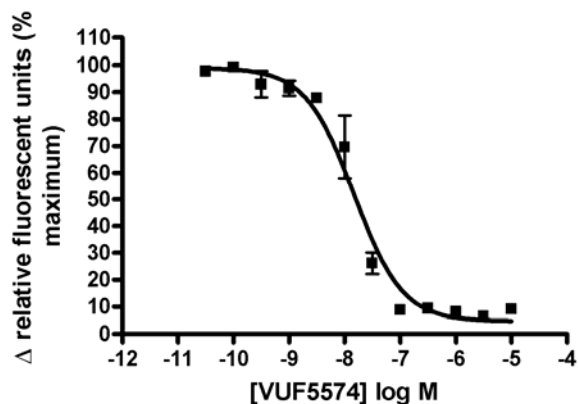


Figure 2. Assay for antagonist activity at A_3 by calcium flux assay. A_3 -expressing Chem-3 cells were loaded with Fluo-4 and washed. VUF5574 was added to the cells at the final concentration indicated, and incubated for 10 min at 37°C. Calcium flux in response to CI-IB-MECA (12 nM) was determined in triplicate on a Molecular Devices FLIPR^{TETRA}™.

SPECIFICATIONS: EC50 for calcium mobilization by CI-IB-MECA: 14 nM
 IC50 for VUF5574: 15 nM
 Signal:noise ratio at CI-IB-MECA E_{max} : 434

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 ADORA3 cDNA encoding A_3 (Accession Number: L22607)

PRESENTATION:

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

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 0.1×10^6 and 1×10^6 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.
4. 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°C overnight. Store the vials in liquid nitrogen.

5. 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)

**RECOMMENDATIONS
FOR CALCIUM ASSAY:**

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:

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

REFERENCES:

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

Tilley SL *et al.* (2003) Identification of A_3 receptor- and mast cell-dependent and -independent components of adenosine-mediated airway responsiveness in mice. *J. Immunol.* 2003 Jul 1;171(1):331-7

Zhong H *et al.* (2003) Activation of murine lung mast cells by the adenosine A_3 receptor. *J. Immunol.* 171: 338-45

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HUMAN RECOMBINANT A₃ RECEPTOR**

Product No. HTS052C

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