

## PRODUCT DATASHEET

### Ready-to-Assay™ MRGPRX1 Mas-Related Gene Receptor Frozen Cells

#### CATALOG NUMBER: HTS122RTA

**CONTENTS:** Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

**STORAGE:** Vials are to be stored in liquid N<sub>2</sub>. Media Component at 4°C (-20°C for prolonged storage).

#### BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

The Mas-related gene family (Mrg), also known as SNSR (sensory neuron-specific G protein-coupled receptors), are expressed mainly in the dorsal root ganglia and appear to be involved in nociception and other sensory processes. MRGPRX1/SNSR4 is activated by the neuropeptide BAM22, and it mediates neuronal synaptic transmission and ion channel activity (Lembo et al., 2002; Burstein et al., 2006, Chen and Ikeda, 2004). Cloned human MRGPRX1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant MRGPRX1 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists, and modulators at MRGPRX1.

#### USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

#### WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures  
Not for Animal or Human Consumption

#### GMO

This product contains genetically modified organisms.  
Este producto contiene organismos genéticamente modificados.  
Questo prodotto contiene degli organismi geneticamente modificati.  
Dieses Produkt enthält genetisch modifizierte Organismen.  
Ce produit contient organismes génétiquement des modifiés.  
Dit product bevat genetisch gewijzigde organismen.  
Tämä tuote sisältää geneettisesti muutettuja organismeja.  
Denna produkt innehåller genetiskt ändrade organismer.

## APPLICATIONS

Calcium Flux Assays

### APPLICATION DATA

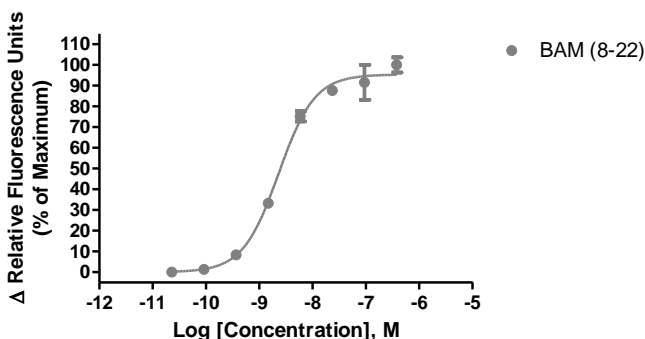


Figure 1. Representative data for activation of MRGPRX1 receptor. Calcium flux in MRGPRX1-expressing Chem-1 cell line induced by BAM (8-22). MRGPRX1-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR<sup>TETRA</sup>. Maximal fluorescence signal obtained in this experiment was 3,650 RLU (Relative Light Units).

Table 1. EC<sub>50</sub> value of MRGPRX1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
BAM (8-22)	Calcium Flux	2.3	Eurofins Internal Data

## ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
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.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37°C 5% CO<sub>2</sub> incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.
10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca<sup>2+</sup> dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca<sup>2+</sup> dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca<sup>2+</sup> dye at 10 µL /10 mL is sufficient for loading one (1) microplate).

11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm (FLIPR<sup>TETRA</sup>) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR<sup>TETRA</sup>) or emission filter for Ca<sup>2+</sup> dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

## ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
BAM (8-22) ligand	Tocris: 1763
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

## FLIPR SETTINGS

Settings for FLIPR<sup>TETRA</sup>® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

## HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

## EXOGENOUS GENE EXPRESSION

MRGPRX1 cDNA (Accession Number: NM\_147199; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

**CODING SEQUENCE**

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1 - ATG GAT CCA ACC ATC TCA ACC TTG GAC ACA GAA CTG ACA CCA ATC AAC GGA ACT GAG GAG ACT CTT TGC TAC - 72
1 - M D P T I S T L D T E L T P I N G T E E T L C Y - 24

73 - AAG CAG ACC TTG AGC CTC ACG GTG CTG ACG TGC ATC GTT TCC CTT GTC GGG CTG ACA GGA AAC GCA GTT GTG - 144
25 - K Q T L S L T V L T C I V S L V G L T G N A V V - 48

145 - CTC TGG CTC CTG GGC TGC CGC ATG CGC AGG AAC GCC TTC TCC ATC TAC ATC CTC AAC TTG GCC GCA GCA GAC - 216
49 - L W L L G C R M R R N A F S I Y I L N L A A A D - 72

217 - TTC CTC TTC CTC AGC GGC CGC CTT ATA TAT TCC CTG TTA AGC TTC ATC AGT ATC CCC CAT ACC ATC TCT AAA - 288
73 - F L F L S G R L I Y S L L S F I S I P H T I S K - 96

289 - ATC CTC TAT CCT GTG ATG ATG TTT TCC TAC TTT GCA GGC CTG AGC TTT CTG AGT GCC GTG AGC ACC GAG CGC - 360
97 - I L Y P V M M F S Y F A G L S F L S A V S T E R - 120

361 - TGC CTG TCC GTC CTG TGG CCC ATC TGG TAC CGC TGC CAC CGC CCC ACA CAC CTG TCA GCG GTG GTG TGT GTC - 432
121 - C L S V L W P I W Y R C H R P T H L S A V V C V - 144

433 - CTG CTC TGG GCC CTG TCC CTG CTG CGG AGC ATC CTG GAA TGG ATG TTA TGT GGC TTC CTG TTC AGT GGT GCT - 504
145 - L L W A L S L L R S I L E W M L C G F L F S G A - 168

505 - GAT TCT GCT TGG TGT CAA ACA TCA GAT TTC ATC ACA GTC GCG TGG CTG ATT TTT TTA TGT GTG GTT CTC TGT - 576
169 - D S A W C Q T S D F I T V A W L I F L C V V L C - 192

577 - GGG TCC AGC CTG GTC CTG CTG ATC AGG ATT CTC TGT GGA TCC CGG AAG ATA CCG CTG ACT AGG CTG TAC GTG - 648
193 - G S S L V L L I R I L C G S R K I P L T R L Y V - 216

649 - ACC ATC CTG CTC ACA GTA CTG GTC TTC CTC CTC TGT GGC CTG CCC TTT GGC ATT CAG TTT TTC CTA TTT TTA - 720
217 - T I L L T V L V F L L C G L P F G I Q F F L F L - 240

721 - TGG ATC CAC GTG GAC AGG GAA GTC TTA TTT TGT CAT GTT CAT CTA GTT TCT ATT TTC CTG TCC GCT CTT AAC - 792
241 - W I H V D R E V L F C H V H L V S I F L S A L N - 264

793 - AGC AGT GCC AAC CCC ATC ATT TAC TTC TTC GTG GGC TCC TTT AGG CAG CGT CAA AAT AGG CAG AAC CTG AAG - 864
265 - S S A N P I I Y F F V G S F R Q R Q N R Q N L K - 288

865 - CTG GTT CTC CAG AGG GCT CTG CAG GAC GCG TCT GAG GTG GAT GAA GGT GGA GGG CAG CTT CCT GAG GAA ATC - 936
289 - L V L Q R A L Q D A S E V D E G G G Q L P E E I - 312

937 - CTG GAG CTG TCG GGA AGC AGA TTG GAG CAG TGA - 969
313 - L E L S G S R L E Q Stp - 322

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**RELATED PRODUCTS**
**PRODUCT NUMBER**
**DESCRIPTION**
**HTSCHEM-1RTA**

Ready-to-Assay™ Chem-1 host frozen cells (control cells)

**HTS122M**

ChemiScreen™ MRGPRX1 Mas-Related receptor membrane prep

**REFERENCES**

1. Wroblowski B *et al.* (2009) The discovery of a selective, small molecule agonist for the MAS-related gene X1 receptor. *J Med Chem.* 52: 818-825.
2. Burstein ES *et al.* (2006) Characterization of the Mas-related gene family: structural and functional conservation of human and rhesus MrgX receptors. *Br J Pharmacol.* 147: 73-82.

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