

PRODUCT DATASHEET

Ready-to-Assay™ CALCRL Calcitonin Receptor Frozen Cells

CATALOG NUMBER: HTS172RTA

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 N₂. 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 calcitonin family of peptides, which includes calcitonin, two calcitonin-related gene peptides (CGRPs), adrenomedullin, and amylin, share a disulfide-bonded loop, an amphipathic α -helix, and an amidated C-terminus. The peptides influence a variety of physiological effects, including vascular tone, food intake and bone metabolism. The biological effects of the peptides are mediated by two class B GPCRs, CT receptor (calcitonin receptor) and CL receptor (calcitonin receptor-like receptor) (Poyner *et al.*, 2002). The pharmacology of the CT and CL receptors is strongly influenced by their association with a family of three receptor activity modifying proteins (RAMPs), which are 14-17 kD single pass transmembrane proteins. CGRP (calcitonin gene-related peptide) induces vasodilation, and its activity is primarily mediated by CGRP₁, composed of CL in complex with RAMP1 (Hasbak *et al.*, 2003). As a consequence of its role as a vasodilator, CGRP acting via CGRP₁ appears to play an important role in hypertension (Deng and Li, 2005). Cloned human CALCRL -expressing cell line is made in the Chem-1 host, which supports high levels of recombinant CALCRL 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, antagonists, and modulators at CALCRL.

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

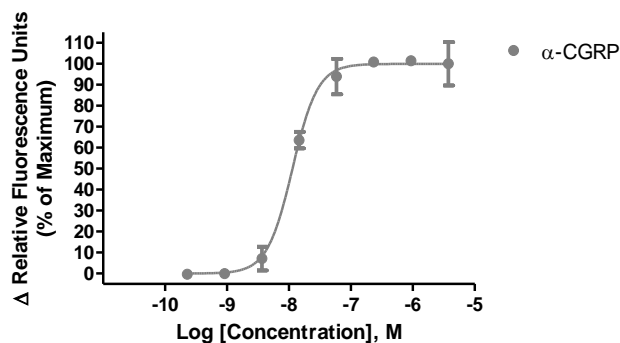


Figure 1. Representative data for activation of CALCRL receptor. Calcium flux in CALCRL-expressing Chem-1 cell line induced by α -CGRP. CALCRL-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 5,350 RLU (Relative Light Units).

Table 1. EC₅₀ value of CALCRL-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
α -CGRP	Calcium Flux	11	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 μ L/well for 96-well plate, 25 μ L/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% CO₂ 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) Ca²⁺ dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca²⁺ dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca²⁺ 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^{TETRA}) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR^{TETRA}) or emission filter for Ca²⁺ 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
Probenecid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
α-CGRP ligand	Bachem: H-1470.0500
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^{TETRA}® 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.

EXONGENOUS GENE EXPRESSION

Human CALCRL cDNA, encoding CGRP₁ and RAMP1 (CGRP₁ Accession Number: NM_005795 see CODING SEQUENCE below; RAMP1 Accession Number: NM_005855) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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atggagaaaaagtgtaccctgtatcttctggttctcttgccttttttatgattctgtt
M E K K C T L Y F L V L L P F F M I L V
acagcagaattagaagagagtcctgaggactcaattcagttgggagttaactagaaataaa
T A E L E E S P E D S I Q L G V T R N K
atcatgacagctcaatatgaatgttaccaaaagattatgcaagacccattcaacaagca
I M T A Q Y E C Y Q K I M Q D P I Q Q A
gaaggcgtttactgcaacagaacctgggatggatggctctgtggaacgatgttcagca
E G V Y C N R T W D G W L C W N D V A A
ggaactgaatcaatgcagctctgtcctgattactttcaggactttgatccatcagaaaaa
G T E S M Q L C P D Y F Q D F D P S E K
gttacaagatctgtgaccaagatggaactggttttagacatccagcaagcaacagaaca
V T K I C D Q D G N W F R H P A S N R T
tggacaattatacccagtgtaatgttaacacccacgagaaagtgaagactgcactaaat
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L F Y L T I I G H G L S I A S L L I S L
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G I F F Y F K S L S C Q R I T L H K N L
ttcttctcatttggtaactctgttgaacaatcattcacctcactgcagtgcccaac
F F S F V C N S V V T I I H L T A V A N
aaccaggccttagtagccacaaaatcctgttagttgcaaagtgccccagttcattcattctt
N Q A L V A T N P V S C K V S Q F I H L
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Y L M G C N Y F W M L C E G I Y L H T L
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S T I F C F F N G E V Q A I L R R N W N
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L N G K S I H D I E N V L L K P E N L Y
aattga
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RELATED PRODUCTS
PRODUCT NUMBER
DESCRIPTION
HTSCHEM-1RTA

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

HTS172M

ChemiScreen™ CALCRL Calcitonin receptor membrane prep

REFERENCES

1. Deng PY and Li YJ (2005) Calcitonin gene-related peptide and hypertension. *Peptides* 26: 1676-85.
2. Hasbak P *et al.* (2003) Investigation of CGRP receptors and peptide pharmacology in human coronary arteries. Characterization with a nonpeptide antagonist. *J. Pharmacol. Exp. Ther.* 304(1):326-33.
3. Poyner DR *et al.* (2002) International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. *Pharmacol. Rev.* 54: 233-246.

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