eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ CaS Calcium Sensor Receptor Frozen Cells

CATALOG NUMBER: HTS137RTA

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_2. Media Component at 4ºC (-20ºC 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.

Calcium homeostasis in vertebrates is regulated by hormonal communication between the parathyroid and thyroid glands, kidney, bone, and intestine. A central mediator of this regulatory system is the calcium sensor CaS (also known as CaR), a class III GPCR that is activated by high concentrations of extracellular calcium and other divalent cations. The cell types that are responsive to variations in blood calcium concentration express CaS and respond to changes in extracellular calcium in a CaS-dependent fashion. Activation of CaS in parathyroid chief cells inhibits PTH release, which results in decreased calcium mobilization from bone. In contrast, stimulation of CaS activity in the C cells of the thyroid increases secretion of calcitonin, which causes decreased bone resorption and increased urinary excretion of calcium. Allosteric "calcimimetic" potentiators are being evaluated for treatment of primary hyperparathyroidism (Brown and MacLeod, 2001). Cloned human CaS-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant CaS expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at CaS Receptor.

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.

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

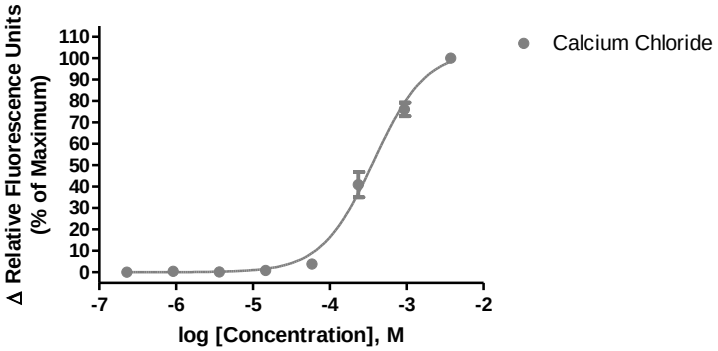

Figure 1. Representative data for activation of CaS receptor. Calcium flux in CaS–expressing Chem-1 cell line induced by calcium chloride. CaS–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 4,500 RLU (Relative Light Units).

Table 1. Comparison of EC_{50} values of CaS-expressing Chem-1 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (µM)	REFERENCE
Calcium Chloride	Calcium Flux	370	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 37ºC 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 37ºC 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) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} 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 Ca^{2+} 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
Calcium Chloride ligand	Sigma: C1016
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

CASR cDNA (Accession Number: NM_000388; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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ATG GCA TTT TAT AGC TGC TGC TGG GTC CTC TTG GCA CTC ACC TGG CAC ACC TCT GCC CAC GGG CCA GAC
 M   A   F   Y   S   C   C   W   V   L   L   A   L   T   W   H   T   S   A   H   G   P   D

CAG CGA GCC CAA AAG AAG GGG GAC ATT ATC CTT GGG GGG CTC TTT CCT ATT CAT TTT GGA GTA GCA GCT
 Q   R   A   Q   K   K   G   D   I   I   L   G   G   L   F   P   I   H   F   G   V   A   A

AAA GAT CAA GAT CTC AAA TCA AGG CCG GAG TCT GTG GAA TGT ATC AGG TAT AAT TTC CGT GGG TTT CGC
 K   D   Q   D   L   K   S   R   P   E   S   V   E   C   I   R   Y   N   F   R   G   F   R

TGG TTA CAG GCT ATG ATA TTT GCC ATA GAG GAG ATA AAC AGC AGC CCA GCC CTT CTT CCC AAC TTG ACG
 W   L   Q   A   M   I   F   A   I   E   E   I   N   S   S   P   A   L   L   P   N   L   T

CTG GGA TAC AGG ATA TTT GAC ACT TGC AAC ACC GTT TCT AAG GCC TTG GAA GCC ACC CTG AGT TTT GTT
 L   G   Y   R   I   F   D   T   C   N   T   V   S   K   A   L   E   A   T   L   S   F   V

GCT CAA AAC AAA ATT GAT TCT TTG AAC CTT GAT GAG TTC TGC AAC TGC TCA GAG CAC ATT CCC TCT ACG
 A   Q   N   K   I   D   S   L   N   L   D   E   F   C   N   C   S   E   H   I   P   S   T

ATT GCT GTG GTG GGA GCA ACT GGC TCA GGC GTC TCC ACG GCA GTG GCA AAT CTG CTG GGG CTC TTC TAC
 I   A   V   V   G   A   T   G   S   G   V   S   T   A   V   A   N   L   L   G   L   F   Y

ATT CCC CAG GTC AGT TAT GCC TCC TCC AGC AGA CTC CTC AGC AAC AAG AAT CAA TTC AAG TCT TTC CTC
 I   P   Q   V   S   Y   A   S   S   S   R   L   L   S   N   K   N   Q   F   K   S   F   L

CGA ACC ATC CCC AAT GAT GAG CAC CAG GCC ACT GCC ATG GCA GAC ATC ATC GAG TAT TTC CGC TGG AAC
 R   T   I   P   N   D   E   H   Q   A   T   A   M   A   D   I   I   E   Y   F   R   W   N

TGG GTG GGC ACA ATT GCA GCT GAT GAC GAC TAT GGG CGG CCG GGG ATT GAG AAA TTC CGA GAG GAA GCT
 W   V   G   T   I   A   A   D   D   D   Y   G   R   P   G   I   E   K   F   R   E   E   A

GAG GAA AGG GAT ATC TGC ATC GAC TTC AGT GAA CTC ATC TCC CAG TAC TCT GAT GAG GAA GAG ATC CAG
 E   E   R   D   I   C   I   D   F   S   E   L   I   S   Q   Y   S   D   E   E   E   I   Q

CAT GTG GTA GAG GTG ATT CAA AAT TCC ACG GCC AAA GTC ATC GTG GTT TTC TCC AGT GGC CCA GAT CTT
 H   V   V   E   V   I   Q   N   S   T   A   K   V   I   V   V   F   S   S   G   P   D   L

GAG CCC CTC ATC AAG GAG ATT GTC CGG CGC AAT ATC ACG GGC AAG ATC TGG CTG GCC AGC GAG GCC TGG
 E   P   L   I   K   E   I   V   R   R   N   I   T   G   K   I   W   L   A   S   E   A   W

GCC AGC TCC TCC CTG ATC GCC ATG CCT CAG TAC TTC CAC GTG GTT GGC GGC ACC ATT GGA TTC GCT CTG
 A   S   S   S   L   I   A   M   P   Q   Y   F   H   V   V   G   G   T   I   G   F   A   L

AAG GCT GGG CAG ATC CCA GGC TTC CGG GAA TTC CTG AAG AAG GTC CAT CCC AGG AAG TCT GTC CAC AAT
 K   A   G   Q   I   P   G   F   R   E   F   L   K   K   V   H   P   R   K   S   V   H   N

GGT TTT GCC AAG GAG TTT TGG GAA GAA ACA TTT AAC TGC CAC CTC CAA GAA GGT GCA AAA GGA CCT TTA
 G   F   A   K   E   F   W   E   E   T   F   N   C   H   L   Q   E   G   A   K   G   P   L

CCT GTG GAC ACC TTT CTG AGA GGT CAC GAA GAA AGT GGC GAC AGG TTT AGC AAC AGC TCG ACA GCC TTC
 P   V   D   T   F   L   R   G   H   E   E   S   G   D   R   F   S   N   S   S   T   A   F

CGA CCC CTC TGT ACA GGG GAT GAG AAC ATC AGC AGT GTC GAG ACC CCT TAC ATA GAT TAC ACG CAT TTA
 R   P   L   C   T   G   D   E   N   I   S   S   V   E   T   P   Y   I   D   Y   T   H   L

CGG ATA TCC TAC AAT GTG TAC TTA GCA GTC TAC TCC ATT GCC CAC GCC TTG CAA GAT ATA TAT ACC TGC
 R   I   S   Y   N   V   Y   L   A   V   Y   S   I   A   H   A   L   Q   D   I   Y   T   C

TTA CCT GGG AGA GGG CTC TTC ACC AAT GGC TCC TGT GCA GAC ATC AAG AAA GTT GAG GCG TGG CAG GTC
 L   P   G   R   G   L   F   T   N   G   S   C   A   D   I   K   K   V   E   A   W   Q   V

CTG AAG CAC CTA CGG CAT CTA AAC TTT ACA AAC AAT ATG GGG GAG CAG GTG ACC TTT GAT GAG TGT GGT
 L   K   H   L   R   H   L   N   F   T   N   N   M   G   E   Q   V   T   F   D   E   C   G

GAC CTG GTG GGG AAC TAT TCC ATC ATC AAC TGG CAC CTC TCC CCA GAG GAT GGC TCC ATC GTG TTT AAG
 D   L   V   G   N   Y   S   I   I   N   W   H   L   S   P   E   D   G   S   I   V   F   K

GAA GTC GGG TAT TAC AAC GTC TAT GCC AAG AAG GGA GAA AGA CTC TTC ATC AAC GAG GAG AAA ATC CTG
 E   V   G   Y   Y   N   V   Y   A   K   K   G   E   R   L   F   I   N   E   E   K   I   L

TGG AGT GGG TTC TCC AGG GAG GTG CCC TTC TCC AAC TGC AGC CGA GAC TGC CTG GCA GGG ACC AGG AAA
 W   S   G   F   S   R   E   V   P   F   S   N   C   S   R   D   C   L   A   G   T   R   K

GGG ATC ATT GAG GGG GAG CCC ACC TGC TGC TTT GAG TGT GTG GAG TGT CCT GAT GGG GAG TAT AGT GAT
 G   I   I   E   G   E   P   T   C   C   F   E   C   V   E   C   P   D   G   E   Y   S   D
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GAG ACA GAT GCC AGT GCC TGT AAC AAG TGC CCA GAT GAC TTC TGG TCC AAT GAG AAC CAC ACC TCC TGC
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ATT GCC AAG GAG ATC GAG TTT CTG TCG TGG ACG GAG CCC TTT GGG ATC GCA CTC ACC CTC TTT GCC GTG
 I   A   K   E   I   E   F   L   S   W   T   E   P   F   G   I   A   L   T   L   F   A   V

CTG GGC ATT TTC CTG ACA GCC TTT GTG CTG GGT GTG TTT ATC AAG TTC CGC AAC ACA CCC ATT GTC AAG
 L   G   I   F   L   T   A   F   V   L   G   V   F   I   K   F   R   N   T   P   I   V   K

GCC ACC AAC CGA GAG CTC TCC TAC CTC CTC CTC TTC TCC CTG CTC TGC TGC TTC TCC AGC TCC CTG TTC
 A   T   N   R   E   L   S   Y   L   L   L   F   S   L   L   C   C   F   S   S   S   L   F

TTC ATC GGG GAG CCC CAG GAC TGG ACG TGC CGC CTG CGC CAG CCG GCC TTT GGC ATC AGC TTC GTG CTC
 F   I   G   E   P   Q   D   W   T   C   R   L   R   Q   P   A   F   G   I   S   F   V   L

TGC ATC TCA TGC ATC CTG GTG AAA ACC AAC CGT GTC CTC CTG GTG TTT GAG GCC AAG ATC CCC ACC AGC
 C   I   S   C   I   L   V   K   T   N   R   V   L   L   V   F   E   A   K   I   P   T   S

TTC CAC CGC AAG TGG TGG GGG CTC AAC CTG CAG TTC CTG CTG GTT TTC CTC TGC ACC TTC ATG CAG ATT
 F   H   R   K   W   W   G   L   N   L   Q   F   L   L   V   F   L   C   T   F   M   Q   I

GTC ATC TGT GTG ATC TGG CTC TAC ACC GCG CCC CCC TCA AGC TAC CGC AAC CAG GAG CTG GAG GAT GAG
 V   I   C   V   I   W   L   Y   T   A   P   P   S   S   Y   R   N   Q   E   L   E   D   E

ATC ATC TTC ATC ACG TGC CAC GAG GGC TCC CTC ATG GCC CTG GGC TTC CTG ATC GGC TAC ACC TGC CTG
 I   I   F   I   T   C   H   E   G   S   L   M   A   L   G   F   L   I   G   Y   T   C   L

CTG GCT GCC ATC TGC TTC TTC TTT GCC TTC AAG TCC CGG AAG CTG CCG GAG AAC TTC AAT GAA GCC AAG
 L   A   A   I   C   F   F   F   A   F   K   S   R   K   L   P   E   N   F   N   E   A   K

TTC ATC ACC TTC AGC ATG CTC ATC TTC TTC ATC GTC TGG ATC TCC TTC ATT CCA GCC TAT GCC AGC ACC
 F   I   T   F   S   M   L   I   F   F   I   V   W   I   S   F   I   P   A   Y   A   S   T

TAT GGC AAG TTT GTC TCT GCC GTA GAG GTG ATT GCC ATC CTG GCA GCC AGC TTT GGC TTG CTG GCG TGC
 Y   G   K   F   V   S   A   V   E   V   I   A   I   L   A   A   S   F   G   L   L   A   C

ATC TTC TTC AAC AAG ATC TAC ATC ATT CTC TTC AAG CCA TCC CGC AAC ACC ATC GAG GAG GTG CGT TGC
 I   F   F   N   K   I   Y   I   I   L   F   K   P   S   R   N   T   I   E   E   V   R   C

AGC ACC GCA GCT CAC GCT TTC AAG GTG GCT GCC CGG GCC ACG CTG CGC CGC AGC AAC GTC TCC CGC AAG
 S   T   A   A   H   A   F   K   V   A   A   R   A   T   L   R   R   S   N   V   S   R   K

CGG TCC AGC AGC CTT GGA GGC TCC ACG GGA TCC ACC CCC TCC TCC TCC ATC AGC AGC AAG AGC AAC AGC
 R   S   S   S   L   G   G   S   T   G   S   T   P   S   S   S   I   S   S   K   S   N   S

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 E   D   P   F   P   Q   P   E   R   Q   K   Q   Q   Q   P   L   A   L   T   Q   Q   E   Q

CAG CAG CAG CCC CTG ACC CTC CCA CAG CAG CAA CGA TCT CAG CAG CAG CCC AGA TGC AAG CAG AAG GTC
 Q   Q   Q   P   L   T   L   P   Q   Q   Q   R   S   Q   Q   Q   P   R   C   K   Q   K   V

ATC TTT GGC AGC GGC ACG GTC ACC TTC TCA CTG AGC TTT GAT GAG CCT CAG AAG AAC GCC ATG GCC CAC
 I   F   G   S   G   T   V   T   F   S   L   S   F   D   E   P   Q   K   N   A   M   A   H

AGG AAT TCT ACG CAC CAG AAC TCC CTG GAG GCC CAG AAA AGC AGC GAT ACG CTG ACC CGA CAC CAG CCA
 R   N   S   T   H   Q   N   S   L   E   A   Q   K   S   S   D   T   L   T   R   H   Q   P

TTA CTC CCG CTG CAG TGC GGG GAA ACG GAC TTA GAT CTG ACC GTC CAG GAA ACA GGT CTG CAA GGA CCT
 L   L   P   L   Q   C   G   E   T   D   L   D   L   T   V   Q   E   T   G   L   Q   G   P

GTG GGT GGA GAC CAG CGG CCA GAG GTG GAG GAC CCT GAA GAG TTG TCC CCA GCA CTT GTA GTG TCC AGT
 V   G   G   D   Q   R   P   E   V   E   D   P   E   E   L   S   P   A   L   V   V   S   S

TCA CAG AGC TTT GTC ATC AGT GGT GGA GGC AGC ACT GTT ACA GAA AAC GTA GTG AAT TCA TAA
 S   Q   S   F   V   I   S   G   G   G   S   T   V   T   E   N   V   V   N   S  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)

REFERENCES

1. Brown EM and MacLeod RJ (2001) Extracellular Calcium Sensing and Extracellular Calcium Signaling. *Physiol. Rev.* 81: 239-297.

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