eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ CaS Calcium Sensor Receptor Stable Cell Line

CATALOG NUMBER: HTS137C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

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 calciuum. Allosteric "calcimimetic" potentiators are being evaluated for treatment of primary hyperparathyroidism (Brown and MacLeod, 2001). The 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 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 antagonists of interactions between CaS and its ligands.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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 Fluorescence Assay

APPLICATION DATA

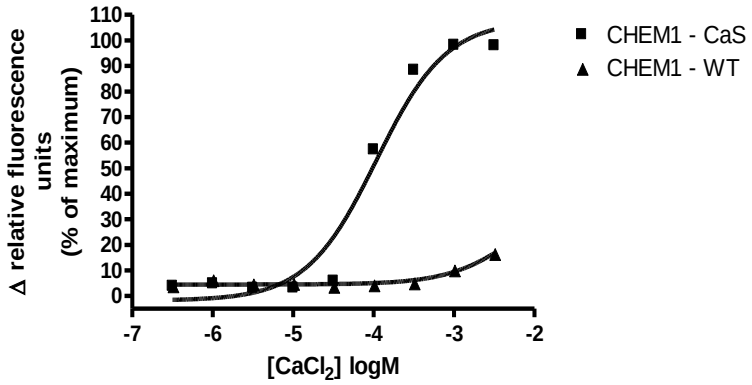

Figure 1. Representative data for activation of CaS receptor stably expressed in Chem-1 cells induced by $CaCl_2$ using a fluorescent calcium flux assay. CaS–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 6,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of CaS-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
$CaCl_2$	Calcium Flux - Fluorescence	11	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP

Fluorescence

Table 4. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
$CaCl_2$	Sigma: C1016
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human CaS cDNA (Accession Number: NM_000388; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

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     69
 M   A   F   Y   S   C   C   W   V   L   L   A   L   T   W   H   T   S   A   H   G   P   D     23

CAG CGA GCC CAA AAG AAG GGG GAC ATT ATC CTT GGG GGG CTC TTT CCT ATT CAT TTT GGA GTA GCA GCT    138
 Q   R   A   Q   K   K   G   D   I   I   L   G   G   L   F   P   I   H   F   G   V   A   A     46

AAA GAT CAA GAT CTC AAA TCA AGG CCG GAG TCT GTG GAA TGT ATC AGG TAT AAT TTC CGT GGG TTT CGC    207
 K   D   Q   D   L   K   S   R   P   E   S   V   E   C   I   R   Y   N   F   R   G   F   R     69

TGG TTA CAG GCT ATG ATA TTT GCC ATA GAG GAG ATA AAC AGC AGC CCA GCC CTT CTT CCC AAC TTG ACG    276
 W   L   Q   A   M   I   F   A   I   E   E   I   N   S   S   P   A   L   L   P   N   L   T     92

CTG GGA TAC AGG ATA TTT GAC ACT TGC AAC ACC GTT TCT AAG GCC TTG GAA GCC ACC CTG AGT TTT GTT    345
 L   G   Y   R   I   F   D   T   C   N   T   V   S   K   A   L   E   A   T   L   S   F   V    115

GCT CAA AAC AAA ATT GAT TCT TTG AAC CTT GAT GAG TTC TGC AAC TGC TCA GAG CAC ATT CCC TCT ACG    414
 A   Q   N   K   I   D   S   L   N   L   D   E   F   C   N   C   S   E   H   I   P   S   T    138

ATT GCT GTG GTG GGA GCA ACT GGC TCA GGC GTC TCC ACG GCA GTG GCA AAT CTG CTG GGG CTC TTC TAC    483
 I   A   V   V   G   A   T   G   S   G   V   S   T   A   V   A   N   L   L   G   L   F   Y    161

ATT CCC CAG GTC AGT TAT GCC TCC TCC AGC AGA CTC CTC AGC AAC AAG AAT CAA TTC AAG TCT TTC CTC    552
 I   P   Q   V   S   Y   A   S   S   S   R   L   L   S   N   K   N   Q   F   K   S   F   L    184

CGA ACC ATC CCC AAT GAT GAG CAC CAG GCC ACT GCC ATG GCA GAC ATC ATC GAG TAT TTC CGC TGG AAC    621
 R   T   I   P   N   D   E   H   Q   A   T   A   M   A   D   I   I   E   Y   F   R   W   N    207

TGG GTG GGC ACA ATT GCA GCT GAT GAC GAC TAT GGG CGG CCG GGG ATT GAG AAA TTC CGA GAG GAA GCT    690
 W   V   G   T   I   A   A   D   D   D   Y   G   R   P   G   I   E   K   F   R   E   E   A    230

GAG GAA AGG GAT ATC TGC ATC GAC TTC AGT GAA CTC ATC TCC CAG TAC TCT GAT GAG GAA GAG ATC CAG    759
 E   E   R   D   I   C   I   D   F   S   E   L   I   S   Q   Y   S   D   E   E   E   I   Q    253

CAT GTG GTA GAG GTG ATT CAA AAT TCC ACG GCC AAA GTC ATC GTG GTT TTC TCC AGT GGC CCA GAT CTT    828
 H   V   V   E   V   I   Q   N   S   T   A   K   V   I   V   V   F   S   S   G   P   D   L    276

GAG CCC CTC ATC AAG GAG ATT GTC CGG CGC AAT ATC ACG GGC AAG ATC TGG CTG GCC AGC GAG GCC TGG    897
 E   P   L   I   K   E   I   V   R   R   N   I   T   G   K   I   W   L   A   S   E   A   W    299

GCC AGC TCC TCC CTG ATC GCC ATG CCT CAG TAC TTC CAC GTG GTT GGC GGC ACC ATT GGA TTC GCT CTG    966
 A   S   S   S   L   I   A   M   P   Q   Y   F   H   V   V   G   G   T   I   G   F   A   L    322

AAG GCT GGG CAG ATC CCA GGC TTC CGG GAA TTC CTG AAG AAG GTC CAT CCC AGG AAG TCT GTC CAC AAT   1035
 K   A   G   Q   I   P   G   F   R   E   F   L   K   K   V   H   P   R   K   S   V   H   N    345

GGT TTT GCC AAG GAG TTT TGG GAA GAA ACA TTT AAC TGC CAC CTC CAA GAA GGT GCA AAA GGA CCT TTA   1104
 G   F   A   K   E   F   W   E   E   T   F   N   C   H   L   Q   E   G   A   K   G   P   L    368

CCT GTG GAC ACC TTT CTG AGA GGT CAC GAA GAA AGT GGC GAC AGG TTT AGC AAC AGC TCG ACA GCC TTC   1173
 P   V   D   T   F   L   R   G   H   E   E   S   G   D   R   F   S   N   S   S   T   A   F    391

CGA CCC CTC TGT ACA GGG GAT GAG AAC ATC AGC AGT GTC GAG ACC CCT TAC ATA GAT TAC ACG CAT TTA   1242
 R   P   L   C   T   G   D   E   N   I   S   S   V   E   T   P   Y   I   D   Y   T   H   L    414

CGG ATA TCC TAC AAT GTG TAC TTA GCA GTC TAC TCC ATT GCC CAC GCC TTG CAA GAT ATA TAT ACC TGC   1311
 R   I   S   Y   N   V   Y   L   A   V   Y   S   I   A   H   A   L   Q   D   I   Y   T   C    437

TTA CCT GGG AGA GGG CTC TTC ACC AAT GGC TCC TGT GCA GAC ATC AAG AAA GTT GAG GCG TGG CAG GTC   1380
 L   P   G   R   G   L   F   T   N   G   S   C   A   D   I   K   K   V   E   A   W   Q   V    460

CTG AAG CAC CTA CGG CAT CTA AAC TTT ACA AAC AAT ATG GGG GAG CAG GTG ACC TTT GAT GAG TGT GGT   1449
 L   K   H   L   R   H   L   N   F   T   N   N   M   G   E   Q   V   T   F   D   E   C   G    483

GAC CTG GTG GGG AAC TAT TCC ATC ATC AAC TGG CAC CTC TCC CCA GAG GAT GGC TCC ATC GTG TTT AAG   1518
 D   L   V   G   N   Y   S   I   I   N   W   H   L   S   P   E   D   G   S   I   V   F   K    506

GAA GTC GGG TAT TAC AAC GTC TAT GCC AAG AAG GGA GAA AGA CTC TTC ATC AAC GAG GAG AAA ATC CTG   1587
 E   V   G   Y   Y   N   V   Y   A   K   K   G   E   R   L   F   I   N   E   E   K   I   L    529

TGG AGT GGG TTC TCC AGG GAG GTG CCC TTC TCC AAC TGC AGC CGA GAC TGC CTG GCA GGG ACC AGG AAA   1656
 W   S   G   F   S   R   E   V   P   F   S   N   C   S   R   D   C   L   A   G   T   R   K    552

GGG ATC ATT GAG GGG GAG CCC ACC TGC TGC TTT GAG TGT GTG GAG TGT CCT GAT GGG GAG TAT AGT GAT   1725
 G   I   I   E   G   E   P   T   C   C   F   E   C   V   E   C   P   D   G   E   Y   S   D    575

GAG ACA GAT GCC AGT GCC TGT AAC AAG TGC CCA GAT GAC TTC TGG TCC AAT GAG AAC CAC ACC TCC TGC   1794
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 E   T   D   A   S   A   C   N   K   C   P   D   D   F   W   S   N   E   N   H   T   S   C     598

ATT GCC AAG GAG ATC GAG TTT CTG TCG TGG ACG GAG CCC TTT GGG ATC GCA CTC ACC CTC TTT GCC GTG   1863
 I   A   K   E   I   E   F   L   S   W   T   E   P   F   G   I   A   L   T   L   F   A   V     621

CTG GGC ATT TTC CTG ACA GCC TTT GTG CTG GGT GTG TTT ATC AAG TTC CGC AAC ACA CCC ATT GTC AAG   1932
 L   G   I   F   L   T   A   F   V   L   G   V   F   I   K   F   R   N   T   P   I   V   K     644

GCC ACC AAC CGA GAG CTC TCC TAC CTC CTC CTC TTC TCC CTG CTC TGC TGC TTC TCC AGC TCC CTG TTC   2001
 A   T   N   R   E   L   S   Y   L   L   L   F   S   L   L   C   C   F   S   S   S   L   F     667

TTC ATC GGG GAG CCC CAG GAC TGG ACG TGC CGC CTG CGC CAG CCG GCC TTT GGC ATC AGC TTC GTG CTC   2070
 F   I   G   E   P   Q   D   W   T   C   R   L   R   Q   P   A   F   G   I   S   F   V   L     690

TGC ATC TCA TGC ATC CTG GTG AAA ACC AAC CGT GTC CTC CTG GTG TTT GAG GCC AAG ATC CCC ACC AGC   2139
 C   I   S   C   I   L   V   K   T   N   R   V   L   L   V   F   E   A   K   I   P   T   S     713

TTC CAC CGC AAG TGG TGG GGG CTC AAC CTG CAG TTC CTG CTG GTT TTC CTC TGC ACC TTC ATG CAG ATT   2208
 F   H   R   K   W   W   G   L   N   L   Q   F   L   L   V   F   L   C   T   F   M   Q   I     736

GTC ATC TGT GTG ATC TGG CTC TAC ACC GCG CCC CCC TCA AGC TAC CGC AAC CAG GAG CTG GAG GAT GAG   2277
 V   I   C   V   I   W   L   Y   T   A   P   P   S   S   Y   R   N   Q   E   L   E   D   E     759

ATC ATC TTC ATC ACG TGC CAC GAG GGC TCC CTC ATG GCC CTG GGC TTC CTG ATC GGC TAC ACC TGC CTG   2346
 I   I   F   I   T   C   H   E   G   S   L   M   A   L   G   F   L   I   G   Y   T   C   L     782

CTG GCT GCC ATC TGC TTC TTC TTT GCC TTC AAG TCC CGG AAG CTG CCG GAG AAC TTC AAT GAA GCC AAG   2415
 L   A   A   I   C   F   F   F   A   F   K   S   R   K   L   P   E   N   F   N   E   A   K     805

TTC ATC ACC TTC AGC ATG CTC ATC TTC TTC ATC GTC TGG ATC TCC TTC ATT CCA GCC TAT GCC AGC ACC   2484
 F   I   T   F   S   M   L   I   F   F   I   V   W   I   S   F   I   P   A   Y   A   S   T     828

TAT GGC AAG TTT GTC TCT GCC GTA GAG GTG ATT GCC ATC CTG GCA GCC AGC TTT GGC TTG CTG GCG TGC   2553
 Y   G   K   F   V   S   A   V   E   V   I   A   I   L   A   A   S   F   G   L   L   A   C     851

ATC TTC TTC AAC AAG ATC TAC ATC ATT CTC TTC AAG CCA TCC CGC AAC ACC ATC GAG GAG GTG CGT TGC   2622
 I   F   F   N   K   I   Y   I   I   L   F   K   P   S   R   N   T   I   E   E   V   R   C     874

AGC ACC GCA GCT CAC GCT TTC AAG GTG GCT GCC CGG GCC ACG CTG CGC CGC AGC AAC GTC TCC CGC AAG   2691
 S   T   A   A   H   A   F   K   V   A   A   R   A   T   L   R   R   S   N   V   S   R   K     897

CGG TCC AGC AGC CTT GGA GGC TCC ACG GGA TCC ACC CCC TCC TCC TCC ATC AGC AGC AAG AGC AAC AGC   2760
 R   S   S   S   L   G   G   S   T   G   S   T   P   S   S   S   I   S   S   K   S   N   S     920

GAA GAC CCA TTC CCA CAG CCC GAG AGG CAG AAG CAG CAG CAG CCG CTG GCC CTA ACC CAG CAA GAG CAG   2829
 E   D   P   F   P   Q   P   E   R   Q   K   Q   Q   Q   P   L   A   L   T   Q   Q   E   Q     943

CAG CAG CAG CCC CTG ACC CTC CCA CAG CAG CAA CGA TCT CAG CAG CAG CCC AGA TGC AAG CAG AAG GTC   2898
 Q   Q   Q   P   L   T   L   P   Q   Q   Q   R   S   Q   Q   Q   P   R   C   K   Q   K   V     966


ATC TTT GGC AGC GGC ACG GTC ACC TTC TCA CTG AGC TTT GAT GAG CCT CAG AAG AAC GCC ATG GCC CAC   2967
 I   F   G   S   G   T   V   T   F   S   L   S   F   D   E   P   Q   K   N   A   M   A   H     989

AGG AAT TCT ACG CAC CAG AAC TCC CTG GAG GCC CAG AAA AGC AGC GAT ACG CTG ACC CGA CAC CAG CCA   3036
 R   N   S   T   H   Q   N   S   L   E   A   Q   K   S   S   D   T   L   T   R   H   Q   P    1012

TTA CTC CCG CTG CAG TGC GGG GAA ACG GAC TTA GAT CTG ACC GTC CAG GAA ACA GGT CTG CAA GGA CCT   3105
 L   L   P   L   Q   C   G   E   T   D   L   D   L   T   V   Q   E   T   G   L   Q   G   P    1035

GTG GGT GGA GAC CAG CGG CCA GAG GTG GAG GAC CCT GAA GAG TTG TCC CCA GCA CTT GTA GTG TCC AGT   3174
 V   G   G   D   Q   R   P   E   V   E   D   P   E   E   L   S   P   A   L   V   V   S   S    1058

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-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS137M	ChemiScreen™ CaS Calcium Sensor Receptor Membrane Prep

REFERENCES

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

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