Description

Recombinant HEK293 cell line stably expressing full-length human Calcitonin receptor-like receptor (CALCRL/CRLR/CLR; accession number: NM_005795) and containing a firefly luciferase gene under the control of multimerized cAMP response element (CRE). This cell line can be used to measure the EC50 and IC50 of CALCRL agonists and antagonists using the luciferase reporter activity.

Background

Calcitonin gene-related peptide (CGRP) receptor is a G-protein coupled receptor (GPCR) with three components: calcitonin receptor-like receptor (CALCRL), receptor activity-modifying protein (RAMP1) and CGRP-receptor component protein (CRCP). RAMP1 is required for both the trafficking of the calcitonin-like receptor to the plasma membrane and the coupling of the receptor with CGRP agonist. CRCP is involved in the association between the CGRP receptor and the G protein. Upon ligand binding, the complex interacts with the G protein, the Gαs subunit dissociates from the complex and activates adenylyl cyclase, which produces cAMP. Elevation of the intracellular concentration of cAMP stimulates cAMP-dependent signaling pathways, ultimately resulting in transcription factor cAMP response element binding protein (CREB) to bind to the CRE promoter and induce gene expression.

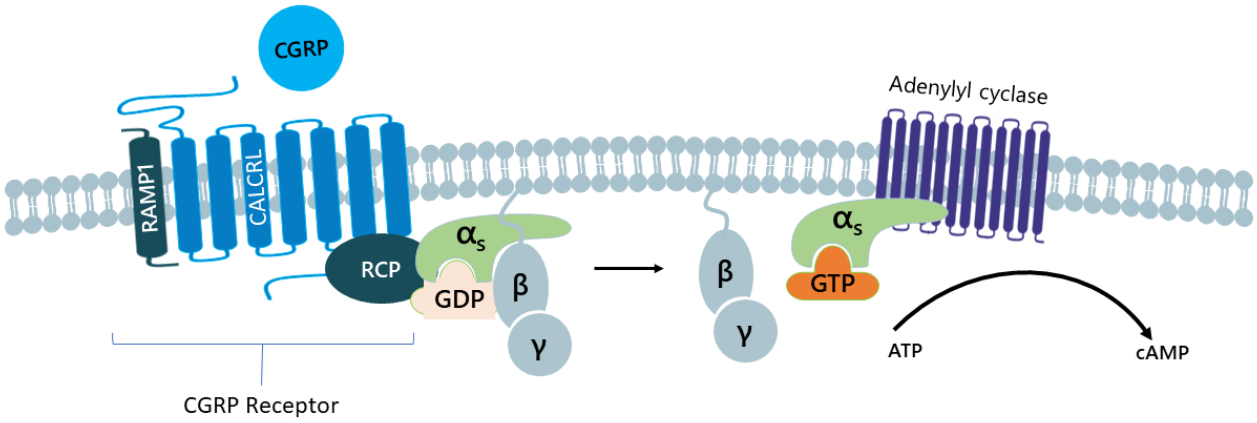

Illustration of CGPR receptor activation at the cell surface

Preclinical evidence suggests that during a migraine, activated sensory neurons in the trigeminal ganglion release CGRP from their projecting nerve endings located within the meninges. The released CGRP then binds and activates CGRP receptors causing vasodilation and plasma extravasation. Further evidence comes from the observation that intravenous administration of α-CGRP induces a headache in susceptible individuals. Recent breakthroughs in understanding the role of CGRP in migraine has led to the development of a novel class of CGRP antagonist biologics that promise significant improvement over conventional pharmacotherapy.

Application

- Screen for activators or inhibitors of CALCRL for immunotherapy research and drug discovery.
- Characterize CALCRL antibodies, antagonists and ligands for binding assay.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of 10% DMSO

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1G	BPS Bioscience #79544

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1G	BPS Bioscience #79544
MEM medium for preparing assay medium	Hyclone #SH30024.01
Human α-CGRP	Sigma #C0167
Rimegepant	Cayman Chemical #26338
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1G (BPS Bioscience #79544):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 400 µg/ml of Geneticin and 50 µg/ml of Hygromycin B.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1 (**no Geneticin or Hygromycin**). **Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.**
2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1 (**no Geneticin or Hygromycin**).
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 (**no Geneticin or Hygromycin**), and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1G (**contains Geneticin and Hygromycin**).

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
1. Once the cells have detached, add Growth Medium 1G and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1G (**contains Geneticin and Hygromycin**). Seed into new culture vessels at the desired sub-cultivation ratio of 1:10 to 1:20 weekly or twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1G and count the cells.

3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.
4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
5. Transfer the vials to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

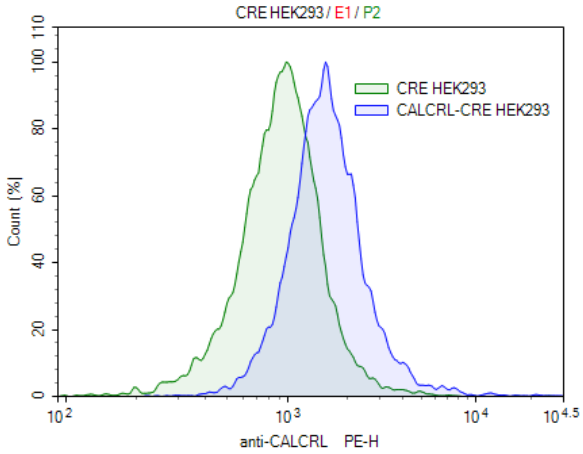

Figure 1: Cell surface expression of human CALCRL in CALCRL/CRE Luciferase Reporter HEK293 cells. CALCRL/CRE Luciferase Reporter HEK293 cells or CRE Luciferase Reporter HEK293 parental cells were stained with both primary anti-CALCRL antibody (Aviva, #APR42260) and PE-labeled anti-rabbit secondary antibody (Biolegend, #406421), and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

B: Functional characterization of CALCRL/CRE Luciferase Reporter HEK293 Cell Line

The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.

a. Protocol for agonist titration assay:

1. Seed CALCRL/CRE Luciferase Reporter HEK293 cells at a density of ~35,000 cells per well into a white clear-bottom 96-well plate in 90 µl of Thaw Medium. 1 Keep three wells without cells for determination of background luminescence.
2. Incubate the plate at 37°C in a CO_2 incubator for 16 to 24 hours.
3. Prepare three-fold serial dilutions of α-GCRP agonist in Thaw Medium 1. For an EC50 dose curve, we recommend a range of approximately 0.005 to 100 nM, final concentration.
   Each condition should be performed in triplicate.

- Add 10 µl of diluted α-GCRP to the treated wells.
- Add 10 µl of Thaw Medium 1 to the "no agonist" control wells.
- Add 100 µl of Thaw Medium 1 to cell-free control wells (for determining background luminescence).

The final incubation volume is 100 µl.

4. Incubate the plate at 37°C in a CO_2 incubator for 4-5 hours.
5. Perform the luciferase assay using the ONE-Step™ Luciferase Assay System as per recommendations. Briefly:
   - Thaw Luciferase Reagents ahead of time. Equilibrate Component A at room temperature and mix well before use.
   - Calculate the amount of Luciferase Reagent needed for the experiment (Component A + Component B). Immediately before use, add 1 volume of Component B to 99 volumes of Component A (1:100 ratio) and mix well. Avoid exposing to excessive light. Store the remaining Component A and Component B separately at -20°C.

   Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at room temperature for 15-30 minutes. Measure luminescence using a luminometer.
6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

   Fold induction of CRE luciferase activity = luminescence of test sample / luminescence of unstimulated control wells (no agonist).

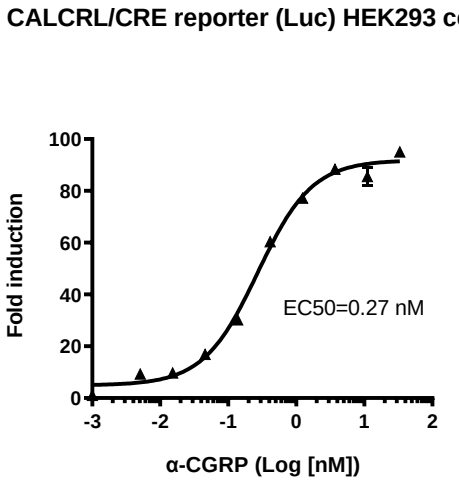

Figure 2. Dose response of agonist α-GCRP in CALCRL/CRE Luciferase Reporter HEK293 cells. α-GCRP ligand (Sigma, #C0167) was serially diluted and added to the cells, for 4-5 hours at 37°C in the cell culture incubator. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). The agonist activated CRE-dependent luciferase activity in a dose-dependent manner.

b. Protocol for antagonist assay:

1. Seed CALCRL/CRE Luciferase Reporter HEK293 cells at a density of ~35,000 cells per well into a white clear-bottom 96-well plate in 80 µl of Thaw Medium 1. Keep three wells without cells for determination of background luminescence.
2. Incubate the plate at 37°C in a CO_2 incubator for 16 to 24 hours.
3. Antagonist preincubation: prepare three-fold serial dilutions of 10 times concentrated (compared to desired final concentration) antagonist Rimegepant (Cayman Chemical, #26338) in Thaw Medium 1. For an IC50 dose curve, we recommend a range of approximately 0.005 to 100 nM, final concentration. Each condition should be performed in triplicate.

   - Add 10 µl of serially diluted Rimegepant to the treated wells.
   - Add 10 µl of Thaw Medium 1 to control "no antagonist" wells.
   - Add 20 µl of Thaw Medium 1 to internal control "no antagonist and no agonist" wells.
   - Add 100 µl of Thaw Medium 1 to cell-free control wells (for determining background luminescence).

4. After 1-hour of preincubation with the antagonist, add 10 µl of 10nM α-GCRP agonist (final 1 nM) to all the treated wells, including the "no antagonist" wells, but NOT in the internal control wells
5. The final incubation volume is 100 µl, incubate the plate at 37°C in a CO_2 incubator for 4-5 hours
6. Perform the luciferase assay using the ONE-Step™ Luciferase Assay System as per recommendations. Briefly:

   - Thaw Luciferase Reagents ahead of time. Equilibrate Component A at room temperature and mix well before use.
   - Calculate the amount of Luciferase Reagent needed for the experiment (Component A + Component B). Immediately before use, add 1 volume of Component B to 99 volumes of Component A (1:100 ratio) and mix well. Avoid exposing to excessive light. Store the remaining Component A and Component B separately at -20°C.

   Add 100 µl of One-Step™ Luciferase reagent to each well and rock at room temperature for 15-30 minutes. Measure luminescence using a luminometer.
7. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.
   Fold induction of CRE luciferase activity = luminescence of test sample / luminescence of unstimulated control wells (no agonist).

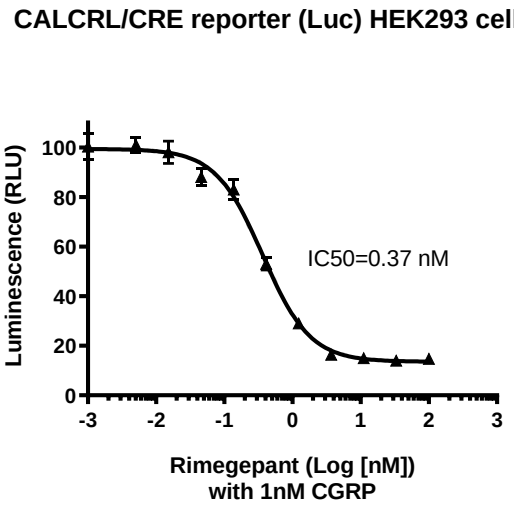

Figure 3. Dose response of antagonist Rimegepant in CALCRL/CRE Luciferase Reporter HEK293 cells. Cells were treated with increasing concentrations of antagonist-Rimegepant (Cayman Chemical, #26338) for 1 hour before addition of α-GCRP agonist (Sigma, #C0167) at a final concentration of 1 nM. Cells were incubated at 37°C in the cell culture incubator for 4-5 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Rimegepant inhibited α-GCRP-induced luciferase activity in dose-dependent manner.

Sequence

Human CALCRL sequence (accession number: NM_005795)

MLYSIFHFGLMMEKKCTLYFLVLLPFFMILVTAELEESPEDSIQLGVTRNKIMTAQYECYQKIMQDPIQQAEGVYCNRTWDGWLC
WNDVAAGTESMQLCPDYFQDFDPSEKVTKICDQDGNWFRHPASNRTWTNYTQCNVNTHEKVKTALNLFYLTIIGHGLSIASLLI
SLGIFFYFKSLSCQRITLHKNLFFSFVCNSVVTIIHLTAVANNQALVATNPVSCKVSQFIHLYLMGCNYFWMLCEGIYLHTLIVVAVF
AEKQHLMWYYFLGWGFPLIPACIHAIARSLYYNDNCWISSDTHLLYIIHGPICAALLVNLFFLLNIVRVLITKLKVTHQAESNLYMKA
VRATLILVPLLGIEFVLIPWRPEGKIAEEVYDYIMHILMHFQGLLVSTIFCFFNGEVQAILRRNWNQYKIQFGNSFSNSEALRSASYT
VSTISDGPGYSHDCPSEHLNGKSIHDIENVLLKPENLYN

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
CRE/CREB Reporter (Luc) – HEK293 Cell Line	60515	2 vials
ONE-Step™ Luciferase assay system	60690	Multiple Sizes
CRE/CREB Reporter (Luc) - Jurkat Cell Line (cAMP/PKA Signaling Pathway)	79636	2 vials
CRE/CREB Luciferase Reporter Lentivirus	79580	500 µl x 2