Description

Recombinant HEK293 cell line stably expressing full-length human calcitonin receptor-like receptor (CALCRL/CRLR/CLR; accession number: NM_005795) and firefly luciferase under the control of a multimerized cAMP response element (CRE). This cell line can be used to measure the EC_{50} and IC_{50} of CGRP receptor agonists and antagonists using luciferase reporter activity as read-out.

Background

Calcitonin gene-related peptide (CGRP) receptor is a G-protein coupled receptor 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.

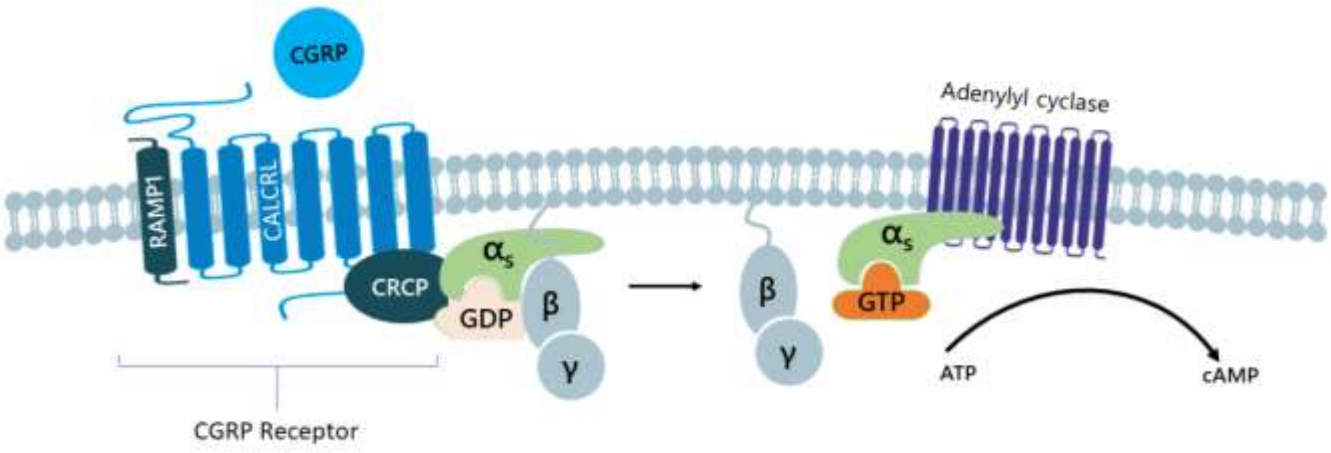

Figure 1: Mechanism of action of CGRP.

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 the CGRP receptor in immunotherapy research and drug discovery.
- Screen for anti-CGRP antibodies.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

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	Hyclone #SH30024.01
Bovine Serum Albumin (BSA, protease free)	Sigma #A4919
Human α-CGRP	Sigma #C0167
Human β- CGRP	Cayman Chemical #24725
Rimegepant	Cayman Chemical #26338
Zavegepant (Vazegepant) Hydrochloride	
Eptinezumab	Invitrogen #MA5-42119
Galcanezumab	Invitrogen #MA5-42106
Anti-CALCRL antibody	Aviva #APR42260
PE Donkey Anti-Rabbit Antibody	Biolegend #406421
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, the use of validated and optimized media from BPS Bioscience *is highly recommended*. 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 to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 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.

Media Required for Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Assay Medium: MEM + 1% BSA (protease free).

Cell Culture Protocol
Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 1 to the conical tube containing the cells. Thaw Medium 1 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. 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.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to be split.

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1G.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1G and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1G.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 to 1:20 weekly or twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, 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 Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. 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 long term storage.

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

Validation Data

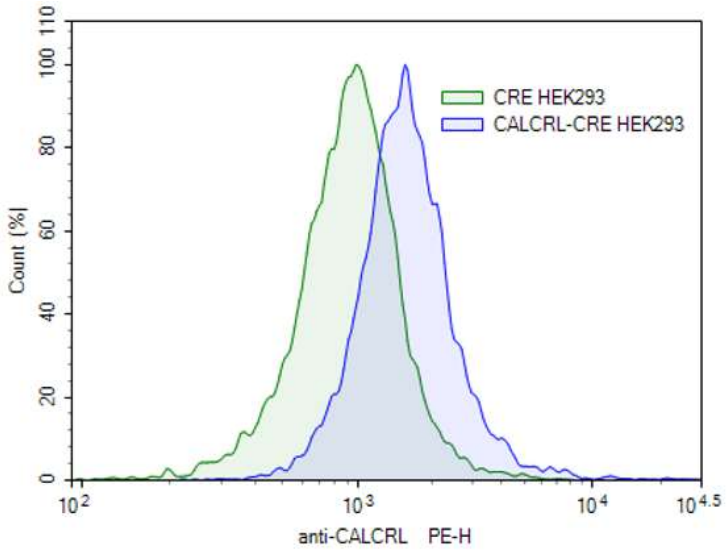

Figure 2: Cell surface expression of human CALCRL in CGRPR/CRE Luciferase Reporter HEK293 Cell Line.
CGRPR/CRE Luciferase Reporter HEK293 cells or CRE Luciferase Reporter HEK293 parental cells were stained with a primary anti-CALCRL antibody (Aviva #APR42260) and PE-labeled donkey anti-rabbit secondary antibody (Biolegend #406421) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates the intensity of PE.

Functional Validation

- 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.
- The assay should be performed in triplicate.
- The assay should include "cell-free" wells and "untreated (no agonist)" wells as controls.
- Antagonist testing should also include "no antagonist and no agonist" wells as control.

Assay medium: MEM + 1% BSA (protease free).

A. Dose response of CGRPR/CRE Luciferase Reporter HEK293 Cell Line to CGRP agonists

1. Seed CGRPR/CRE Luciferase Reporter HEK293 cells at a density of ~35,000 cells per well into a white clear-bottom 96-well plate in 100 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. Carefully remove the Thaw Medium 1 from the plate and replace with 50 µl of Assay Medium.

HEK293 cells are easily detached during the medium change. Use a pipette to slowly remove the medium from the wells. Do not use an aspirator. Some degree of detachment should not affect the results if well-to-well variations are not significant.

4. Prepare serial dilutions of α-CGRP or β-CGRP agonists at concentrations 2-fold higher than the desired final concentrations in Assay Medium. For an EC_{50} dose curve, we recommend a dose range of approximately 0.001 to 100 nM.

5. Add 50 µl of diluted CGRP agonists to the treated wells.

6. Add 50 µl of Assay Medium to the "untreated (no agonist)" control wells.

7. Add 100 µl of Assay Medium to the "cell-free" control wells (for determining background luminescence).

8. Incubate the plate at 37°C in a CO_2 incubator for 4-5 hours.

9. Add 100 µl of ONE-Step™ Luciferase reagent to each well.

10. Rock at Room Temperature (RT) for ~15 minutes.

11. Measure luminescence using a luminometer.

12. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of CRE luciferase reporter expression is the background-subtracted luminescence of treated well divided by the average background-subtracted luminescence of untreated control wells.

$$Fold\ induction = \frac{average\ Lum\ sample - average\ backgound}{average\ Lum\ control - average\ background}$$

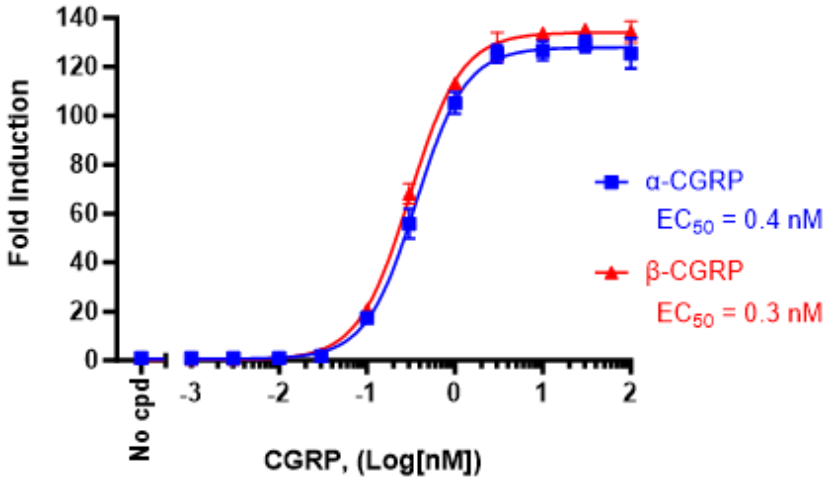

Figure 3: Dose response of CGRPR/CRE Luciferase Reporter HEK293 Cell Line to the agonists α-CGRP and β-CGRP.
CGRPR/CRE Luciferase Reporter HEK293 cells were treated with increasing concentrations of α-CGRP (blue) or β-CGRP (red). The agonists activated CRE-dependent luciferase activity in a dose-dependent manner. Activity was measured with ONE-Step™ Luciferase Assay System. The fold induction is equal to background-subtracted luminescence of antibody-treated well/background-subtracted luminescence of untreated-control.

B. Dose response of CGRPR/CRE Luciferase Reporter HEK293 Cell Line to the CGRP antagonist Rimegepant

1. Seed CGRPR/CRE Luciferase Reporter HEK293 cells at a density of ~35,000 cells per well into a white clear-bottom 96-well plate in 100 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. Carefully remove the Thaw Medium 1 from the plate and replace with 50 µl of Assay Medium.

HEK293 cells are easily detached during the medium change. Use a pipette to slowly remove the medium from the wells. Do not use an aspirator. Some degree of detachment should not affect the results if well-to-well variations are not significant.

4. Prepare Rimegepant dilutions (25 µl/ well): prepare serial dilutions of antagonist Rimegepant at concentrations 4-fold higher than the desired final concentration in Assay Medium.

5. Add 25 µl of serially diluted Rimegepant to the treated wells.

6. Add 25 µl of Assay Medium to the control "untreated (no antagonist)" wells.

7. Add 50 µl of Assay Medium to the control "no antagonist and no agonist" wells.

8. Add 100 µl of Assay Medium to the "cell-free" control wells (for determining background luminescence).

9. Preincubate cells with the antagonist for 1 hour at 37°C in a CO_2 incubator.

10. Add 25 µl of 4 nM α-CGRP or β-CGRP agonists to all treated wells, including the "untreated (no antagonist)" wells, but NOT in the "no antagonist and no agonist" internal control wells.

11. Incubate the plate at 37°C in a CO_2 incubator for 4-5 hours.

12. Add 100 µl of ONE-Step™ Luciferase reagent to each well.

13. Rock at Room Temperature (RT) for ~15 minutes.

14. Measure luminescence using a luminometer.

15. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The Percent luminescence of CRE Luciferase Reporter Activity is the background-subtracted luminescence of the treated wells divided by the average background-subtracted luminescence of the untreated wells, multiplied by 100.

$$\text{Percent Luminescence} = \left(\frac{\text{luminescence treated wells} - \text{background}}{\text{ave luminescence untreated wells} - \text{background}}\right) \text{x100}$$

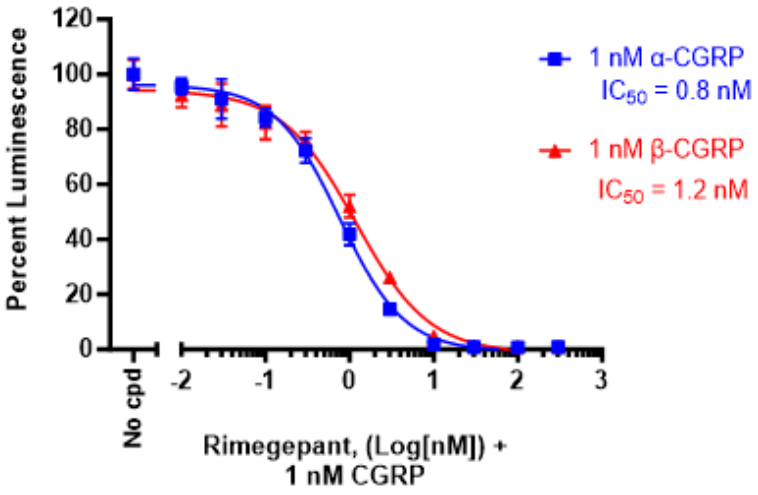

Figure 4: Dose response of CGRPR/CRE Luciferase Reporter HEK293 Cell Line to the small molecule antagonist Rimegepant.
CGRPR/CRE Luciferase Reporter HEK293 cells were treated with increasing concentrations of Rimegepant for 1 hour before addition of α-CGRP agonist (blue) or β-CGRP agonist (red), both at a final concentration of 1 nM. Activity was measured with ONE-Step™ Luciferase Assay System.

C. Dose response of CGRPR/CRE Luciferase Reporter HEK293 Cell Line to the CGRP antagonists Eptinezumab and Galcanezumab

1. Seed CGRPR/CRE Luciferase Reporter HEK293 cells at a density of ~35,000 cells per well into a white clear-bottom 96-well plate in 100 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. Carefully remove the Thaw Medium 1 from the plate and replace with 50 µl of Assay Medium.

HEK293 cells are easily detached during the medium change. Use a pipette to slowly remove the medium from the wells. Do not use an aspirator. Some degree of detachment should not affect the results if well-to-well variations are not significant.

4. Prepare antagonist dilutions (25 µl/well): prepare serial dilutions of the anti-CGRP antibodies Eptinezumab or Galcanezumab, at concentrations 4-fold higher than the desired final concentration in Assay Medium. For an IC_{50} dose curve, we recommend a dose range of approximately 0.0003 to 10 µg/ml.

5. Add 25 µl of serially diluted Eptinezumab or Galcanezumab to 25 µl of 4 nM α-CGRP or β-CGRP agonists.

6. Preincubate for 1 hour at 37°C in a CO_2 incubator.

7. Add 50 µl of antagonist + CGRP mix to the "treated" wells.

8. Add 25 µl of Assay Medium and 25 µl of agonist solution to the "untreated (no antagonist)" control wells.

9. Add 50 µl of Assay Medium to internal control "no antagonist and no agonist" wells.

10. Add 100 µl of Assay Medium to the cell-free control wells (for determining background luminescence).

11. Incubate the plate at 37°C in a CO_2 incubator for 4-5 hours.

12. Add 100 µl of ONE-Step™ Luciferase reagent to each well.

13. Rock at RT for ~15 minutes.

14. Measure luminescence using a luminometer.

15. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The Percent luminescence of CRE Luciferase Reporter Activity is the background-subtracted luminescence of the treated wells divided by the average background-subtracted luminescence of the untreated wells, multiplied by 100.

$$\text{Percent Luminescence} = \left(\frac{\text{luminescence treated wells} - \text{background}}{\text{ave luminescence untreated wells} - \text{background}}\right) \text{x100}$$

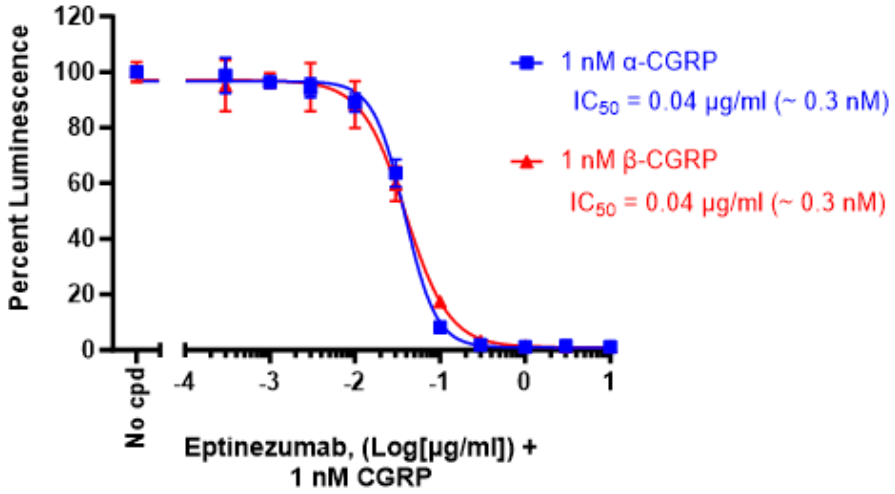

Figure 5: Dose response of CGRPR/CRE Luciferase Reporter HEK293 Cell Line to the anti-CGRP Eptinezumab.
Increasing concentrations of Eptinezumab were pre-incubated with 1 nM α-CGRP agonist (blue) or β-CGRP agonist (red) for 1 hour before addition to cells for 4-5 hours. Activity was measured with ONE-Step™ Luciferase Assay System.

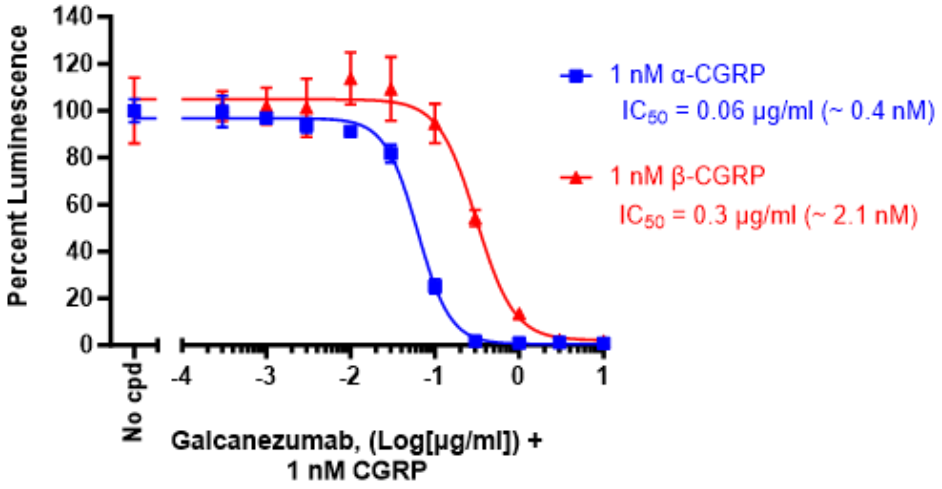

Figure 6: Dose response of CGRPR/CRE Luciferase Reporter HEK293 Cell Line to the anti-CGRP antibody Galcanezumab.
Increasing concentrations of Galcanezumab were pre-incubated with 1 nM α-CGRP agonist (blue) or β-CGRP agonist (red) for 1 hour before addition to cells for 4-5 hours. Activity was measured with ONE-Step™ Luciferase Assay System.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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	10 ml/100 ml/500 ml
CRE/CREB Reporter (Luc) - Jurkat Cell Line (cAMP/PKA Signaling Pathway)	79636	2 vials
CRE/CREB Luciferase Reporter Lentivirus	79580	500 µl x 2