Description

The Amylin Receptor 3 (AMY3R)/CRE Luciferase Reporter HEK293 Cell Line is a HEK293 cell line engineered to express CALCR (calcitonin receptor, NM_001164737.3) and RAMP3 (receptor activity modifying protein 3, NM_005856.3), delivered into CRE/CREB Luciferase Reporter HEK293 Cell Line (cAMP/PKA Signaling Pathway) (#60515), which expresses a firefly luciferase reporter driven by a multimerized cAMP response element (CRE). Activation of the receptor in these cells can be monitored by measuring luciferase activity.

This cell line was validated in dose-response assays using the peptide agonists Amylin, Calcitonin, Pramlintide, Cagrilintide, and Eloralintide.

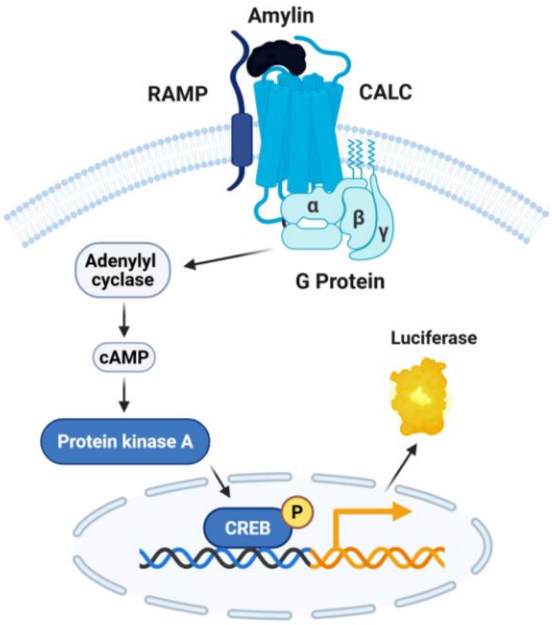

Figure 1: Illustration of the mechanism of action of Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 Cell Line.

Background

Amylin, also known as islet amyloid polypeptide or IAPP, is a peptide hormone produced by pancreatic beta-cells and secreted alongside insulin, downstream of nutrient sensing. Circulating amylin signals through the amylin family of receptors which are comprised of two subunits, the shared G-protein calcitonin receptor (CALCR) and one of three receptor activity-modifying protein subunits (RAMPs 1-3) to generate AMY1R, AMY2R and AMY3R, respectively. The receptors signal downstream through the second messenger cAMP. All three subtypes of the amylin receptor are expressed in brain regions that control feeding and satiety. Amylin action through the AMYRs plays a role in satiety and control of blood glucose levels. Amylin agonists and dual amylin/ calcitonin receptor agonists are currently under development for the treatment of both obesity and type-2 diabetes (T2D).

Application

Screen or titrate Amylin receptor agonists.

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.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1Y	BPS Bioscience # 82535

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Amylin, amide, human TFA	BPS Bioscience #83586
Pramlintide	BPS Bioscience #83587
Eloralintide	BPS Bioscience #83588
Calcitonin	BPS Bioscience #83791
Cagrilintide	BPS Bioscience #83792
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.

Contraindications

- Residual trypsin may impact the stability of the agonists being tested. Make sure to wash cells prior to incubation with agonists.
- The % of DMSO in the assay should not exceed 0.1%.

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˚C 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 1Y (BPS Bioscience # 82535):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 0.5 µg/ml of Puromycin, 100 µg/ml of Hygromycin B, and 400 µg/ml of G418.

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.

Cell Culture Protocol

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

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.

   Note: 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.

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 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 1Y.

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 1Y 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 1Y**.**

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 to 1:8 once or twice per 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 1Y 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.

Functional Validation

- The following assays are designed for 96-well format or 384-well format as specified. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The assay conditions should be performed in triplicate.
- Assays should include “Background Control”, “Unstimulated Control” and “Stimulated” conditions.

Assay Medium:

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

A. Dose response of Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 Cell Line to Amylin Receptor agonists (96 well plate format).

1. Detach Amylin Receptor 3 (AMY3R) cells with 0.05% Trypsin/EDTA, and centrifuge.

2. Wash the cells once with Assay Medium.

   Note: This step is important to remove residual trypsin from the culture, which can impact peptide stability.

3. Seed the cells into a white clear-bottom 96-well microplate at a density of ~30,000 cells per well in 80 µl of Assay Medium. Leave a few wells empty for use as the cell-free control wells ("Background Control").

4. Incubate cells at 37°C in a CO_2 incubator for 16 to 24 hours.

5. The next day, prepare a serial dilution of Amylin agonists in Assay Medium at concentrations 5-fold higher than the desired final concentration (20 µl/well). For peptide agonists it is recommended to use a new pipet tip for each dilution to avoid sample carry over.

   5.1 For agonists soluble in water-based buffers, prepare a serial dilution in Assay Medium at the desired concentrations. Assay Medium is the Diluent Solution.

   OR

   5.2 For agonists soluble in DMSO, such as Pramlintide, prepare a stock solution in 100% DMSO at a concentration 1,000x higher than the highest desired final concentration, then dilute it 1000-fold with Assay Medium to prepare the highest concentration of the serial dilution. The concentration of DMSO is now 0.1% DMSO.

   Prepare a serial dilution at the desired concentrations using Assay Medium containing 0.1% DMSO. For controls use Assay Medium with 0.1% DMSO (Diluent Solution).

   Note: The concentration of DMSO should not exceed 0.1% in the final reaction.

6. Add 20 µl of Amylin Receptor agonist dilutions to the "Stimulated" wells.

7. Add 20 µl of Diluent Solution to the "Unstimulated Control" wells.

8. Add 100 µl of Diluent Solution to the "Background Control" wells (for determining background luminescence).

9. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

10. Add 100 µl of the ONE-Step™ Luciferase reagent per well.

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

12. Measure luminescence using a luminometer.

13. Data analysis: The fold induction of the CRE luciferase reporter is the average luminescence of the stimulated wells divided by the average luminescence of the unstimulated control wells.

Note: Luminescence values of unstimulated cells are typically low in this cell line and can be comparable to the cell-free media controls. For this reason, we do not recommend performing background subtraction as this can result in negative values and distortions of the fold induction.

$$Fold\ induction = \frac{luminescence\ of\ stimulated\ cells}{average\ luminesence\ of\ unstimulated\ cells}$$

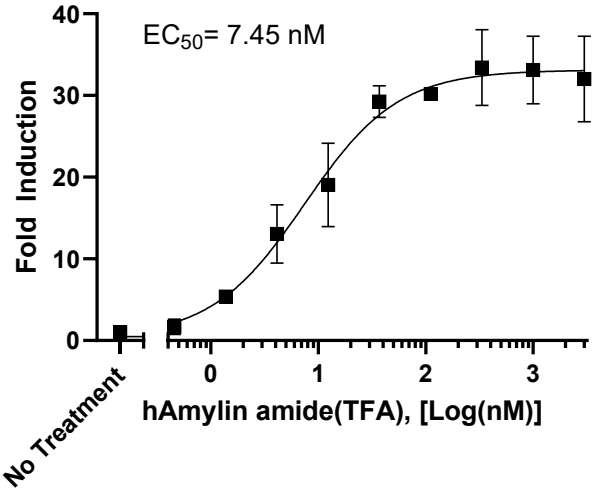

Figure 2: Dose response of Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 Cell Line to human Amylin, amide (TFA).
Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 cells were treated with increasing concentrations of human Amylin amide (TFA) for 5-6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

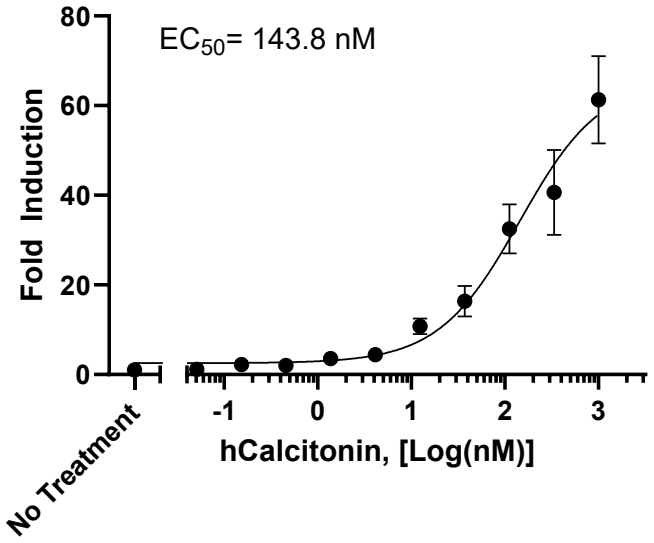

Figure 3: Dose response of Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 Cell Line to human Calcitonin.
Amylin Receptor 3 (AMY3R) /CRE Luciferase Reporter HEK293 cells were treated with increasing concentrations of human Calcitonin for 5-6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

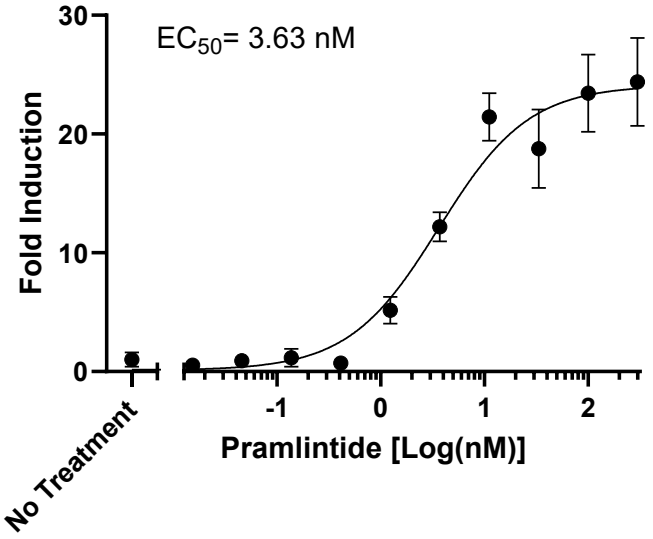

Figure 4: Dose response of Amylin Receptor 3 (AMY3R/) CRE Luciferase Reporter HEK293 Cell Line to Pramlintide.
Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 cells were treated with increasing concentrations of Pramlintide for 5-6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

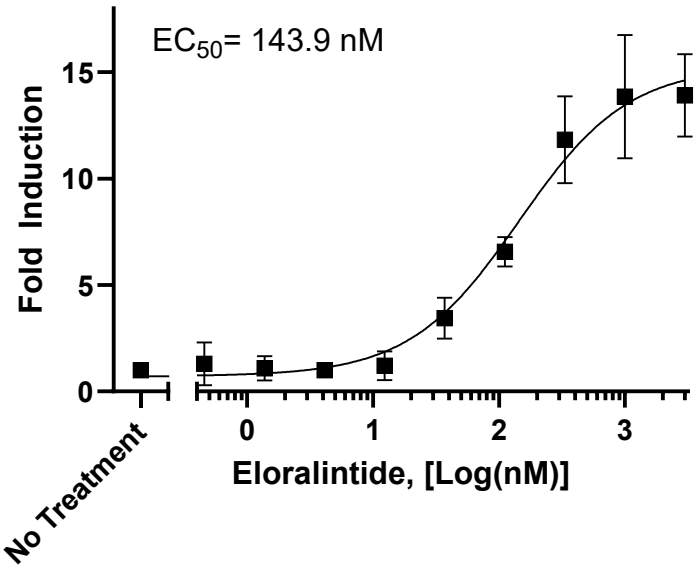

Figure 5: Dose response of Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 Cell Line to Eloralintide.
Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 cells were treated with increasing concentrations of Eloralintide for 5-6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

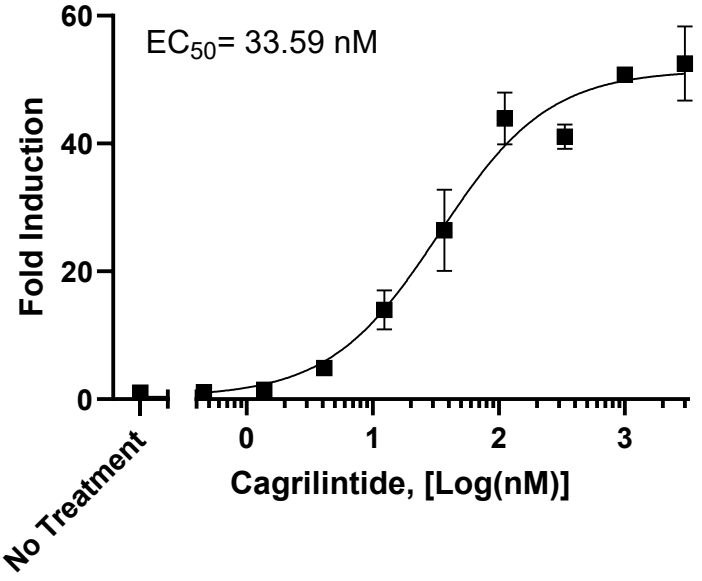

Figure 6: Dose response of Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 Cell Line to Cagrilintide.
Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 cells were treated with increasing concentrations of Cagrilintide for 5-6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus unstimulated control.

B. Dose response of Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 Cell Line to Amylin Receptor agonists (384 well plate format).

1. Detach Amylin Receptor 3 (AMY3R) cells with 0.05% Trypsin/EDTA, and centrifuge.

2. Wash the cells once with Assay Medium.

   Note: This step is important to remove residual trypsin from the culture, which can impact peptide stability.

3. Seed the cells into a white walled/ white-bottom 384-well microplate at a density of ~10,000 cells per well in 20 µl of Assay Medium. Leave a few wells empty for use as the cell-free control wells ("Background Control").

4. Incubate cells at 37°C in a CO_2 incubator for 16 to 24 hours.

5. The next day, prepare a serial dilution of the desired Amylin agonist in Assay Medium at concentrations 5-fold higher than the desired final concentration (5 µl/well). For peptide agonists it is recommended to use a new pipet tip for each dilution to avoid sample carry over.

   5.1 For agonists soluble in water-based buffers, prepare a serial dilution in Assay Medium at the desired concentrations. Assay Medium is the Diluent Solution.

   OR

   5.2 For agonists soluble in DMSO, such as Pramlintide, prepare a stock solution in 100% DMSO at a concentration 1,000x higher than the highest desired final concentration, then dilute it 1000-fold with Assay Medium to prepare the highest concentration of the serial dilution. The concentration of DMSO is now 0.1% DMSO.

   Prepare a serial dilution at the desired concentrations using Assay Medium containing 0.1% DMSO. For controls use Assay Medium with 0.1% DMSO (Diluent Solution).

   Note: The concentration of DMSO should not exceed 0.1% in the final reaction.

6. Add 5 µl of Amylin Receptor agonist dilutions to the "Stimulated" wells.

7. Add 5 µl of Diluent Solution to the "Unstimulated Control" wells.

8. Add 25 µl of Diluent Solution to the "Background Control" wells (for determining background luminescence).

9. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

10. Add 25 µl of the ONE-Step™ Luciferase reagent per well.

11. Rock gently at RT for ~15 minutes.

12. Measure luminescence using a luminometer.

13. Data analysis: The fold induction of the CRE luciferase reporter is the average luminescence of the stimulated wells divided by the average luminescence of the unstimulated control wells.

    Note: Luminescence values of unstimulated cells are typically low in this cell line and can be comparable to the cell-free media controls. For this reason, we do not recommend performing background subtraction as this can result in negative values and distortions of the fold induction.

$$Fold\ induction = \frac{luminescence\ of\ stimulated\ cells}{average\ luminesence\ of\ unstimulated\ cells}$$

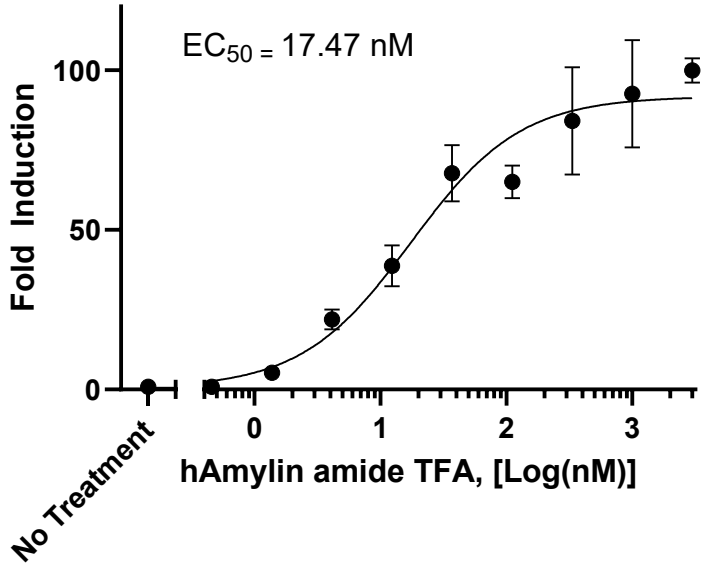

Figure 7: Dose response of Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 Cell Line to human Amylin, amide (TFA) in a 384 well format.
Amylin Receptor 3 (AMY3R)/ CRE Luciferase Reporter HEK293 cells were treated with increasing concentrations of human Amylin amide (TFA) for 5-6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

Data shown is representative.

Sequence

Human CALCR sequence (accession number NM_001164737.3):
MRFTFTSRCLALFLLLNHPTPILPAFSNQTYPTIEPKPFLYVVGRKKMMDAQYKCYDRMQQLPAYQGEGPYCNRTWDGWLCW
DDTPAGVLSYQFCPDYFPDFDPSEKVTKYCDEKGVWFKHPENNRTWSNYTMCNAFTPEKLKNAYVLYYLAIVGHSLSIFTLVISLG
IFVFFRKLTTIFPLNWKYRKALSLGCQRVTLHKNMFLTYILNSMIIIIHLVEVVPNGELVRRDPVSCKILHFFHQYMMACNYFWMLC
EGIYLHTLIVVAVFTEKQRLRWYYLLGWGFPLVPTTIHAITRAVYFNDNCWLSVETHLLYIIHGPVMAALVVNFFFLLNIVRVLVTK
MRETHEAESHMYLKAVKATMILVPLLGIQFVVFPWRPSNKMLGKIYDYVMHSLIHFQGFFVATIYCFCNNEVQTTVKRQWAQF
KIQWNQRWGRRPSNRSARAAAAAAEAGDIPIYICHQELRNEPANNQGEESAEIIPLNIIEQESSA

Human RAMP3 sequence (accession number NM_005856.3):

METGALRRPQLLPLLLLLCGGCPRAGGCNETGMLERLPLCGKAFADMMGKVDVWKWCNLSEFIVYYESFTNCTEMEANVVGC
YWPNPLAQGFITGIHRQFFSNCTVDRVHLEDPPDEVLIPLIVIPVVLTVAMAGLVVWRSKRTDTLL

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
Bower R.L., *et al.,* 2016 *Br J Pharmacol.* 173(12):1883-98
Kruse T., *et al.,* 2021 *Journal of Medicinal Chemistry* 64(15): 11183-11194
Sonne N., *et al.,* 2021 *Mol Metab.* 46:101109
Walker C.S., *et al.,* 2025 *Nat Rev Endocrinol.* 21:482-494

Related Products

Products	*Catalog #*	*Size*
CRE/CREB Luciferase Reporter HEK293 Cell Line (cAMP/PKA Signaling Pathway)	60515	2 vials
Calcitonin Receptor CRE Luciferase Reporter HEK293 Cell Line	83585	2 vials
GLP-1R/CRE Luciferase Reporter HEK293 Cell Line	78176	2 vials
GLP-2R/CRE luciferase Reporter HEK293 Cell Line	83623	2 vials

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