Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell Development Service Data Sheet

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Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell

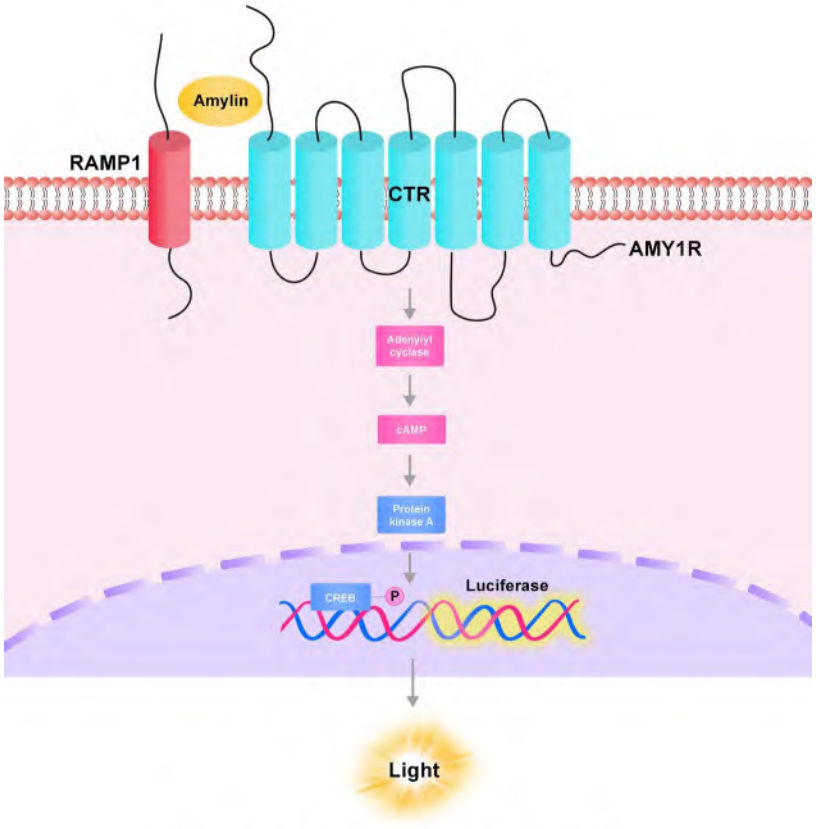
Catalog No.	Size
SCBHK-ATF262	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell was engineered to not only express CREB signaling response element, but also express the human AMY1R generated by co-expressing the full length human calcitonin receptor (CTR) (Uniprot: P30988-2) and the full length human receptor activity-modifying protein 1 (RAMP1) (Uniprot: O60894) added with an mGFP tag, which can drive luciferase expressing systems by amylin receptor (AMYR) agonists or amylin stimulation. In the absence of agonist or amylin, the AMY1R receptor is not activated and luminescence signal is low. In the presence of agonist or amylin, the AMY1R pathway-activated luminescence can be detected in a dose-dependent manner.

• *Application*

- Screen for AMYR agonists that can bind and activate AMY1R.



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• *Cell Line Profile*

Cell line	Human AMY1R (CTR&RAMP1) (Luc)BHK 21 Reporter Cell
Host Cell	BHK 21
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL) + Hygromycin B (20 μg/mL) + Zeocin (20 μg/mL)
Incubation	37°C with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

• *Materials Required for Cell Culture*

- DMEM Medium (BasalMedia, Cat. No. L120KJ)

Note: If you are unable to obtain the specified DMEM medium (BasalMedia, Cat. No. L120KJ), you may use an alternative DMEM medium (Gibco, Cat. No. 11965-092) or another suitable medium for culturing.

- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Puromycin (InvivoGen, Cat. No. ant-pr-5b)
- Hygromycin B (Invitrogen, Cat. No. 10687010)
- Zeocin (Invitrogen, Cat. No. R25001)

Note: For selection antibiotics, we highly recommend using the specified brand. The activity of antibiotics may vary between manufacturers, so if you choose to use a different brand, it is essential to validate whether the concentration recommended in the culture medium is suitable. Regardless of the brand used, we recommend maintaining a backup culture without selection antibiotics to avoid potential cell loss due to inappropriate antibiotic concentration.

- 0.25% Trypsin-EDTA (1X), Phenol Red (Gibco, Cat. No. 25200-056)
- Penicillin-Streptomycin (Gibco, Cat. No. 15140-122)
- Phosphate Buffered Saline (1X) (HyClone, Cat. No. SH30256.01)
- Complete Growth Medium: DMEM + 10% FBS, 1%P/S
- Culture Medium: DMEM + 10% FBS, Puromycin (2 μg/mL), Hygromycin B (20 μg/mL), Zeocin (20 μg/mL), 1%P/S
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, Cat. No. 430641)
- Cryogenic storage vials (SARSTEDT, Cat. No. 72.379.007)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell200A Plus)
- CO_2 Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

• *Recovery*

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize the risk of contamination, ensure the cap remains out of the water. Thawing should be completed quickly, typically within 3-5 minutes.
2. After thawing, promptly remove the vial from the water bath and decontaminate it by spraying with 70% ethanol. From this point onward, all operations must be performed under strict aseptic conditions.
3. Transfer the contents of the vial to a centrifuge tube containing 4.0 mL of complete growth medium. Centrifuge at approximately 1000 rpm for 5 minutes.
4. Resuspend the cell pellet with 5 mL complete growth medium and transfer the cell suspension into a T-75 flask containing 10-15 mL of pre-warmed complete growth medium.
5. Incubate at 37°C with 5% CO_2 incubator until the cells are ready to be split.

• *Subculture*

1. Cell viability may be low after thawing, and full recovery may take up to a week. Monitor the cells daily until the culture reaches 80-90% confluency. At this point, remove and discard the spent medium. Avoid allowing the cells to become over-confluent to ensure optimal cell health.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 4-5 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of culture medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps.
5. Transfer appropriate aliquots of the cell suspension to a new T-75 flask. A subcultivation ratio of 1:6 to 1:10 is recommended. Adjust the ratio based on your specific culture system.
6. Incubate at 37°C with 5% CO_2 incubator.
7. When the cell culture reaches 80-90% confluency, proceed to the next subculture. Avoid over-confluency, as this may negatively impact cell performance in subsequent passages.

Note: After recovery, maintain the cells for 1-2 passages in the complete growth medium not containing the selection marker, if the cells are in good condition, transition to the culture medium containing the selection marker during subculturing.

• *Cryopreservation*

1. When the cell culture reaches 80-90% confluency, remove and discard the spent medium.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 4-5 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of complete growth medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps. Count the viable cells.
5. Transfer the cell suspension to a centrifuge tube. Centrifuge at 1000 rpm for 5 min at room temperature to pellet the cells.
6. After centrifugation, discard the supernatant. Resuspend the cells in ice cold freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
7. Aliquot the cell suspension into cryogenic storage vials. Place the vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transfer to liquid nitrogen storage for long-term storage.

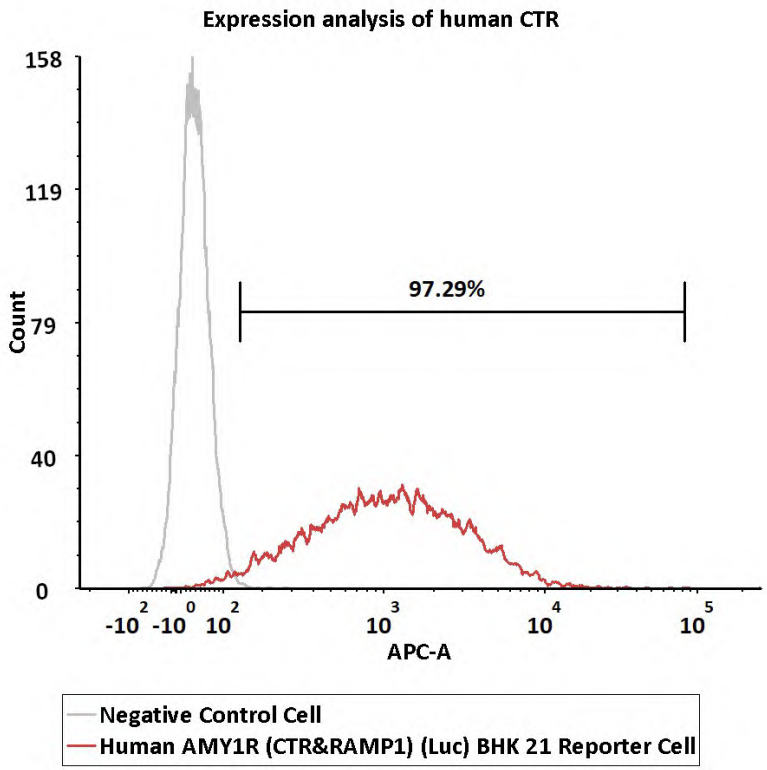
   Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *Storage Condition*

Cells must be received in a frozen state on dry ice and should be transferred to liquid nitrogen or a –80°C freezer immediately upon receipt. If stored in a –80°C freezer, it is recommended to limit the storage period to no more than two weeks. For long-term preservation, transfer the cells to liquid nitrogen is highly recommended.

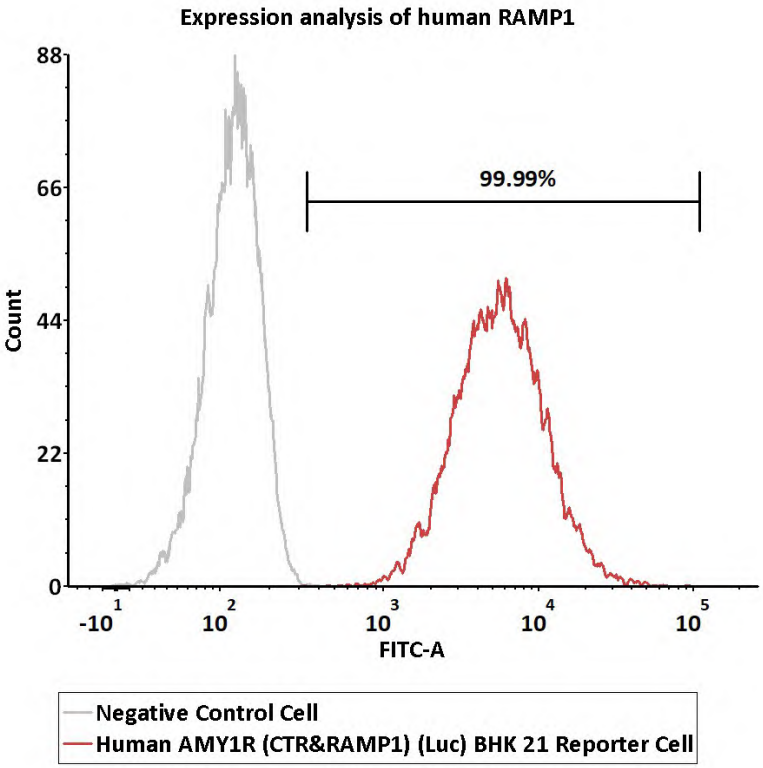
Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell Development Service Data Sheet

- ***Receptor Assay***



Catalog No.	Stable Cell Line	MFI for CTR (APC)
NA	Negative Control Cell	31.31
SCBHK-ATF262	Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell	1015.55

Fig1. Expression analysis of human CTR on Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell by FACS. Cell surface staining was performed on Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell or negative control cell using anti-human CTR antibody followed by staining with APC anti-mouse IgG antibody.



Catalog No.	Stable Cell Line	MFI for RAMP1 (FITC)
NA	Negative Control Cell	118.47
SCBHK-ATF262	Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell	5338.84

Fig2. Expression analysis of human receptor activity-modifying protein 1 (RAMP1) on Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell by FACS. Surface expression of human RAMP1 on Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell was confirmed by detection of mGFP (FITC) using flow cytometry.

• *Signaling Bioassay*

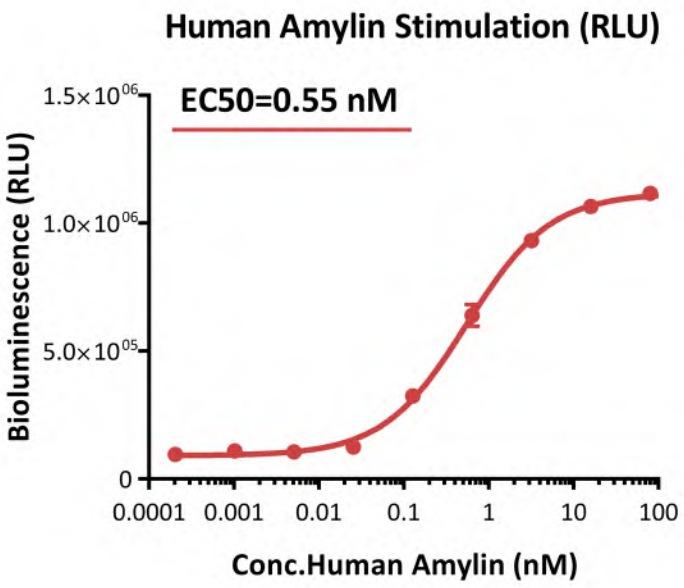


Fig3. Response to human Amylin (RLU). This Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell was incubated with serial dilutions of human Amylin. The EC50 was approximately 0.55 nM.

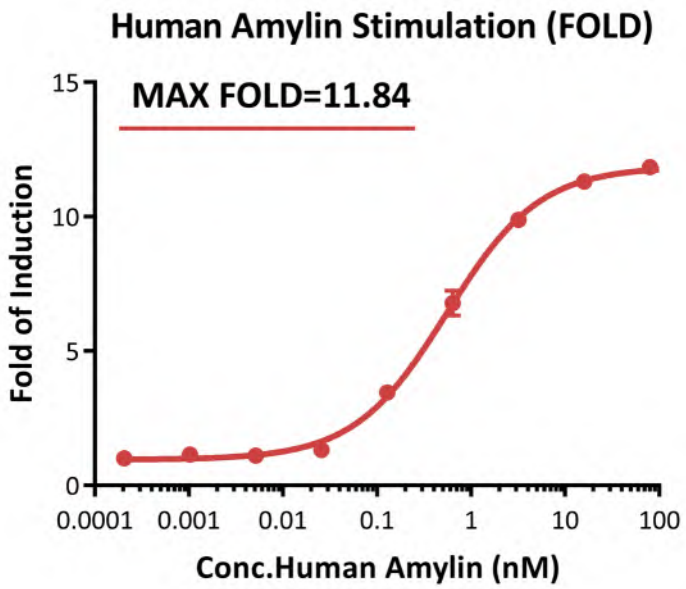


Fig4. Response to human Amylin (FOLD). This Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell was incubated with serial dilutions of human Amylin. The max induction fold was approximately 11.84.

• *Application*

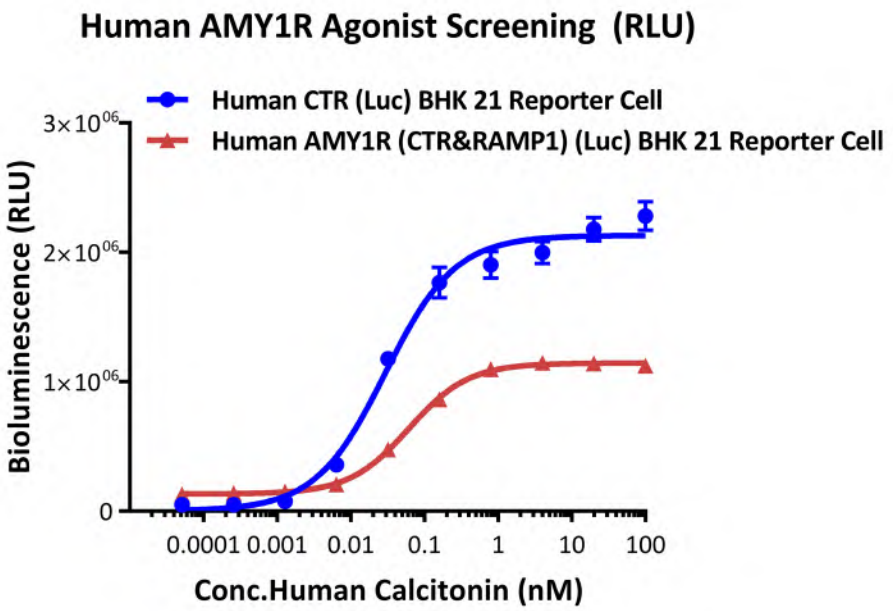


Fig5. Bioactivity analysis of human AMY1R agonist (RLU). The Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell and the parental Human CTR (Luc) BHK 21 Reporter Cell (Cat. No. SCBHK-ATF230) was incubated with serial dilutions of human Calcitonin. The EC50 of human Calcitonin determined on the Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell was approximately 0.06 nM, and on the Human CTR (Luc) BHK 21 Reporter Cell was approximately 0.03 nM. The fold change (EC50 [Human CTR (Luc) BHK 21 Reporter Cell] / EC50 [Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell]) was approximately 0.50, demonstrating that human Calcitonin was non-selective for human AMY1R over human CTR.

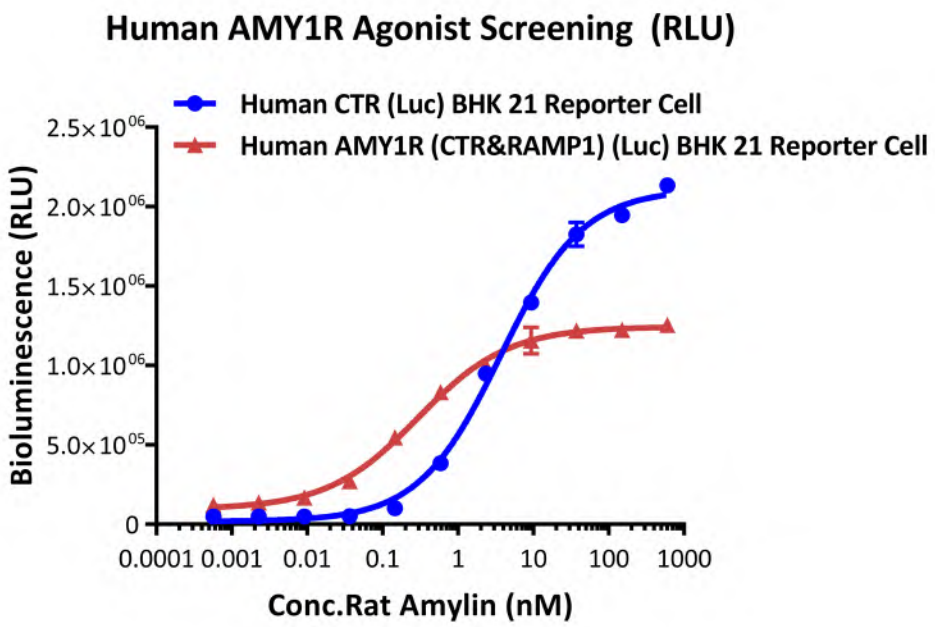


Fig6. Bioactivity analysis of human AMY1R agonist (RLU). The Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell and the parental Human CTR (Luc) BHK 21 Reporter Cell (Cat. No. SCBHK-ATF230) was incubated with serial dilutions of rat Amylin. The EC50 of rat Amylin determined on the Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell was approximately 0.30 nM, and on the Human CTR (Luc) BHK 21 Reporter Cell was approximately 3.70 nM. The fold change (EC50 [Human CTR (Luc) BHK 21 Reporter Cell] / EC50 [Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell]) was approximately 12.33, demonstrating that rat Amylin was selective for human AMY1R over human CTR.

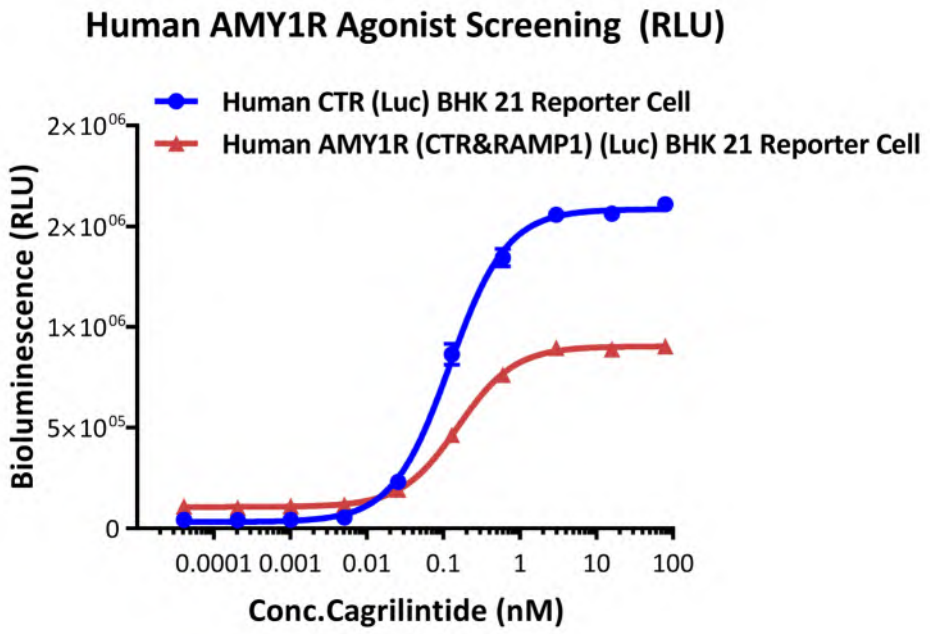
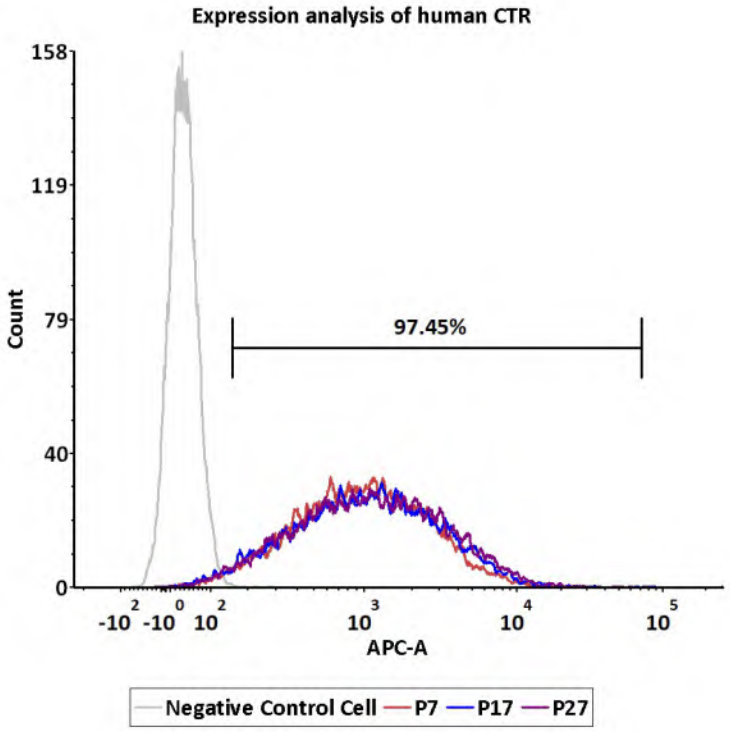


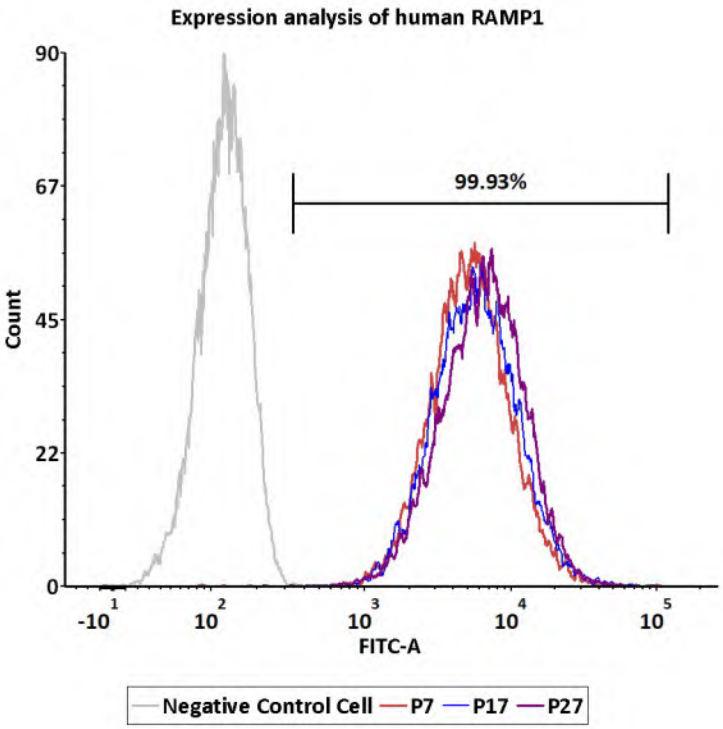
Fig7. Bioactivity analysis of human AMY1R agonist (RLU). The Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell and the parental Human CTR (Luc) BHK 21 Reporter Cell (Cat. No. SCBHK-ATF230) was incubated with serial dilutions of Cagrilintide. The EC50 of Cagrilintide determined on the Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell was approximately 0.16 nM, and on the Human CTR (Luc) BHK 21 Reporter Cell was approximately 0.12 nM. The fold change (EC50 [Human CTR (Luc) BHK 21 Reporter Cell] / EC50 [Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell]) was approximately 0.75, demonstrating that Cagrilintide was non-selective for human AMY1R over human CTR.

• ***Passage Stability***



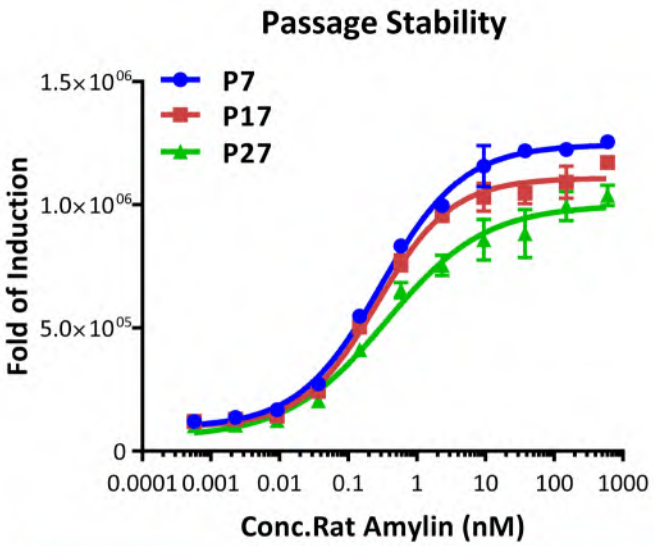
Passage	MFI for CTR (APC)
P7	940.23
P17	1013.01
P27	1052.78

Fig8. Passage stability analysis of human CTR receptor expression by FACS. Flow cytometry surface staining of human CTR on Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell demonstrates consistent mean fluorescent intensity across passage 7-27.



Passage	MFI for RAMP1 (FITC)
P7	5016.06
P17	5431.56
P27	6226.93

Fig9. Passage stability analysis of human RAMP1 receptor expression by FACS. Flow cytometry surface expression of human RAMP1 on Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell demonstrates consistent mean fluorescent intensity across passage 7-27.



	P7	P17	P27
EC50(nM)	0.30	0.27	0.36
Max Fold	11.20	10.68	10.43

Fig10. Passage stability analysis by Signaling Bioassay. The continuously growing Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell was stimulated with serial dilutions of rat Amylin. Rat Amylin stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 7-27.

Human AMY1R (CTR&RAMP1) (Luc) BHK 21 Reporter Cell Development Service Data Sheet

• *Related Products*

Products	Cat.No.
Human AMY3R (CTR&RAMP3) (Luc) BHK 21 Reporter Cell Development Service	SCBHK-ATF231
Human CTR (Luc) BHK 21 Reporter Cell Development Service	SCBHK-ATF230
HEK293/Human GIPR Stable Cell Line (High Expression)	CHEK-ATP206
HEK293/Human GIPR Stable Cell Line (Medium Expression)	CHEK-ATP207
HEK293/Human GIPR Stable Cell Line (Low Expression)	CHEK-ATP208
HEK293/Human GLP-1R Stable Cell Line (High Expression)	CHEK-ATP160
HEK293/Human GLP-1R Stable Cell Line (Medium Expression)	CHEK-ATP161
HEK293/Human GLP-1R Stable Cell Line (Low Expression)	CHEK-ATP162
HEK293/Human ASGR1 Stable Cell Line	CHEK-ATP080
Human GLP-1R (Luc) HEK293 Reporter Cell	CHEK-ATF096
Human GCGR (Luc) HEK293 Reporter Cell	CHEK-ATF103
Human FGF-21 (Luc) HEK293 Reporter Cell	CHEK-ATF163
Human Activin RII (Luc) HEK293 Reporter Cell	CHEK-ATF164
HEK293/Human ASGR1&ASGR2 Stable Cell Line	CHEK-ATP172
HEK293/Human GPR75 Stable Cell Line	CHEK-ATP174
Human THRB (Luc) HEK293 Reporter Cell	CHEK-ATF181
Human THRA (Luc) HEK293 Reporter Cell	CHEK-ATF180
Human GIPR (Luc) HEK293 Reporter Cell	CHEK-ATF104
HEK293/Human GCGR Stable Cell Line (High Expression)	CHEK-ATP209
HEK293/Human GCGR Stable Cell Line (Medium Expression)	CHEK-ATP210
HEK293/Human GCGR Stable Cell Line (Low Expression)	CHEK-ATP211
HEK293/Human LDL R Stable Cell Line	CHEK-ATP158
HEK293/Human GLP-1R&GIPR Stable Cell Line	CHEK-ATP205