Human GCGR (Luc) HEK293 Reporter Cell Data Sheet

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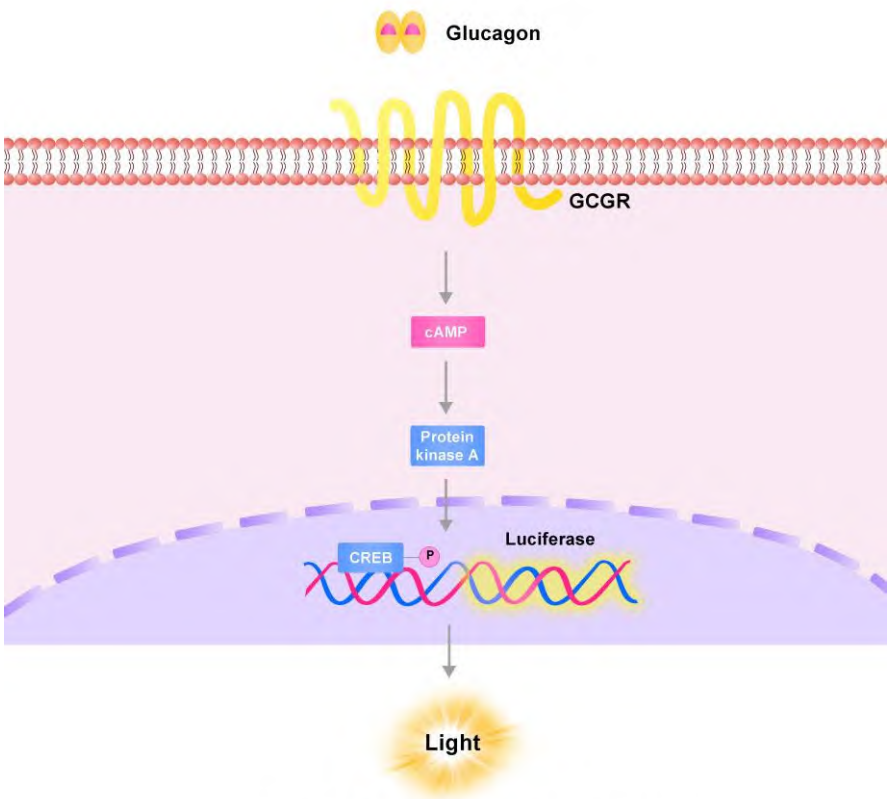
Catalog No.	Size
CHEK-ATF103	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human GCGR (Luc) HEK293 Reporter Cell was engineered to not only express CREB signaling response element, but also express the receptor full length human GCGR (Gene ID: 2642), which can drive luciferase expressing systems by GCGR agonists or Glucagon stimulation. In the absence of agonist or Glucagon, the GCGR receptor is not activated and luminescence signal is low. In the presence of agonist or Glucagon, the GCGR pathway-activated luminescence can be detected in a dose-dependent manner.

• *Application*

- Screen for agonists that can bind and activate GCGR.



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• *Cell Line Profile*

Cell line	Human GCGR (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL) + Hygromycin (20 μg/mL)
Incubation	37℃ with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

• *Materials Required for Cell Culture*

- DMEM medium (Gibco, Cat.No.11965-092)
- Fetal bovine serum (CellMax, Cat.No.SA211.02)
- Puromycin (InvivoGen, Cat.No.ant-pr-5b)
- Hygromycin B (Invitrogen, Cat.No.10687010)
- Complete Growth Medium: DMEM + 10% FBS
- Culture Medium: DMEM + 10% FBS, Puromycin (2 μg/mL), Hygromycin (20 μg/mL)
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, 430641)
- Cryogenic storage vials (SARSTEDT, 72.379.007)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Logos Biosystems, LUNA-Ⅱ)
- CO_2 Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)

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• *Recovery*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 4.0 mL complete growth medium and spin at approximately 1000 rpm for 5 minutes.
4. Resuspend cell pellet with 5 mL complete growth medium and transfer the cell suspension into 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. Remove and discard culture medium.
2. Wash the cells once with sterile PBS.
3. Add 2 mL of 0.25% trypsin to cell culture flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached.
4. Add 6.0 to 8.0 mL of culture medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessel.
6. Incubate at 37°C with 5% CO_2 incubator.

Subcultivation Ratio: A subcultivation ratio of 1:6 to 1:10 is recommended.

Medium Renewal: Every 2 to 3 days.

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• *Cryopreservation*

1. Remove and discard spent medium.
2. Detach cells from the cell culture flasks with 0.25% trypsin.
3. Centrifuge at 1000 rpm for 5 min at RT to pellet cells.
4. Resuspend the cell pellets with complete growth medium and count viable cells.
5. Centrifuge at 1000 rpm for 5 min at RT and resuspend cells in freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
6. Aliquot into cryogenic storage vials. Place vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transferring to liquid nitrogen storage.

• *Storage*

- **Product format:** Frozen
- **Storage conditions:** Liquid nitrogen immediately upon receipt

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- ***Receptor Assay***

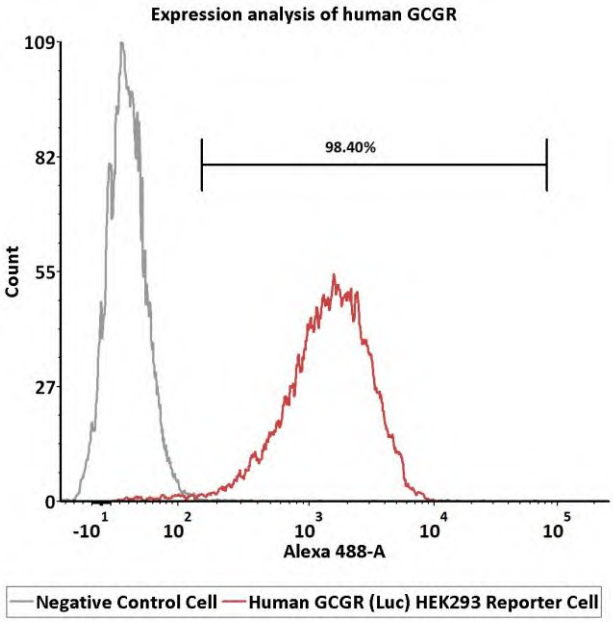

Fig1. Expression analysis of human GCGR on Human GCGR (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human GCGR (Luc) HEK293 Reporter Cell or negative control cell using Alexa Fluor® 488-labeled anti-human GCGR antibody.

- ***Application***

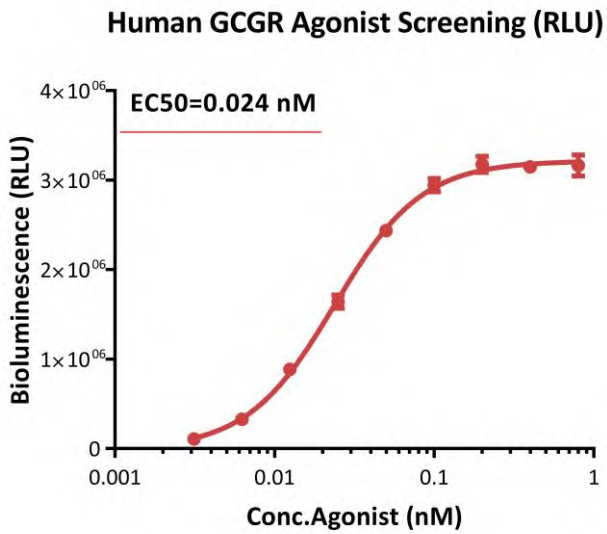

Fig2. Bioactivity analysis of human GCGR agonist (RLU). This reporter cell was incubated with serial dilutions of Retatrutide (a triple agonist peptide of GCGR, GIPR and GLP-1R). The EC50 of Retatrutide was approximately 0.024 nM.

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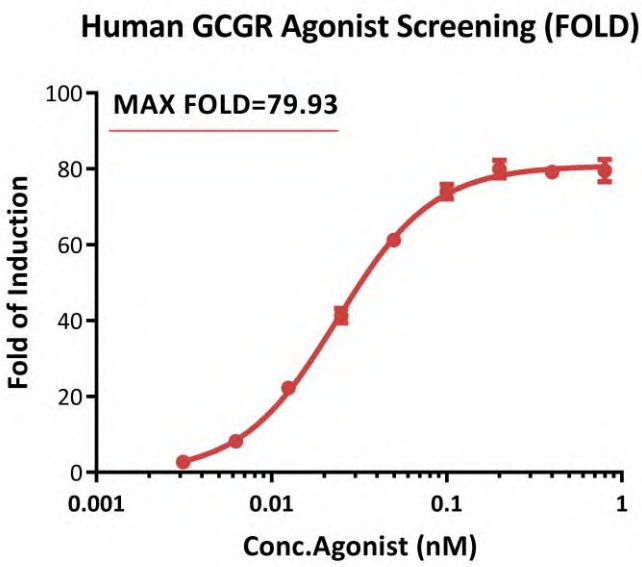

Fig3. Bioactivity analysis of human GCGR agonist (FOLD). This reporter cell was incubated with serial dilutions of Retatrutide (a triple agonist peptide of GCGR, GIPR and GLP-1R). The max induction fold was approximately 79.93.

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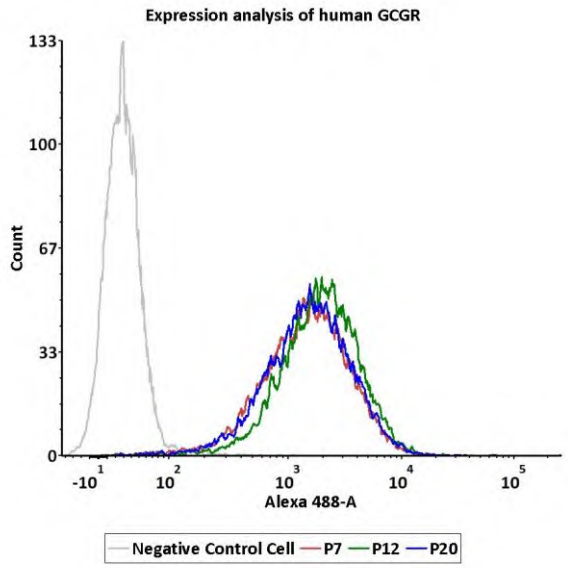
- ***Passage Stability***



Passage	MFI for GCGR (Alexa 488)
P7	1423.50
P12	1865.01
P20	1484.38

Fig4. Passage stability analysis of receptor expression by FACS. Flow cytometry surface staining of human GCGR on Human GCGR (Luc) HEK293 Reporter Cell demonstrates consistent mean fluorescent intensity across passage 7-20.

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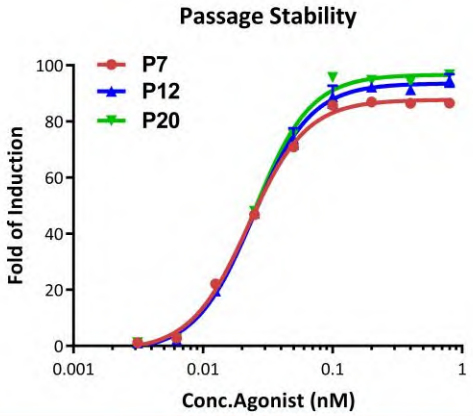

	P7	P12	P20
EC50 (nM)	0.02241	0.02427	0.02467
Max Fold	86.96	94.64	96.61

Fig5. Passage stability analysis by Signaling Bioassay. The continuously growing Human GCGR (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of Retatrutide (a triple agonist peptide of GCGR, GIPR and GLP-1R). Retatrutide stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 7-20.

• *License Disclosure*

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• *Related Products*

Products	Cat.No.
Human GLP-1R (Luc) HEK293 Reporter Cell	CHEK-ATF096
Human GIPR (Luc) HEK293 Reporter Cell	CHEK-ATF104