

## CERTIFICATE OF ANALYSIS

### Product Information

Product Name	HEK 293/GCGR/Gα15
Cat. No.	M00422
Lot No.	B80191710
Host Cell:	HEK293
Target Gene:	GCGR
Quantity:	2 vials of frozen cells
Shipping Condition:	Dry Ice
Recommended Storage Condition:	Liquid Nitrogen

### Stable Cell Line Information

**Recommended Cell Culture Medium:** DMEM + 10% FBS + 300 µg/ml G418 + 50 µg/ml Hygromycin B

**Freeze Medium:** 90%FBS, 10% (V/V) DMSO

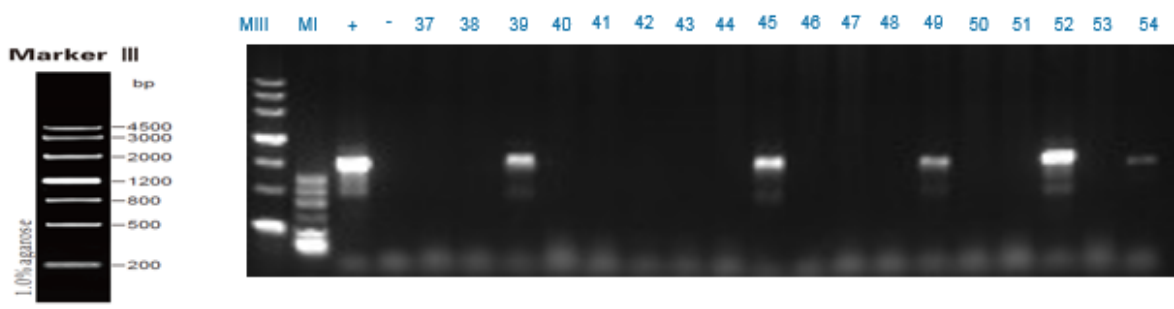
**Application:** Functional assay for HEK 293/GCGR/Gα15

**Note:** The cells should be cultured in cell culture medium without antibiotics first for about 2-3 days after the cell thawing. The antibiotics (G418 and Hygromycin B) will be used when the cells recover.

Test Item	Specification	Result
Mycoplasma 160	Negative.	Negative., Appendix 1
Functional assay	Calcium assay	EC <sub>50</sub> =69.7 nM

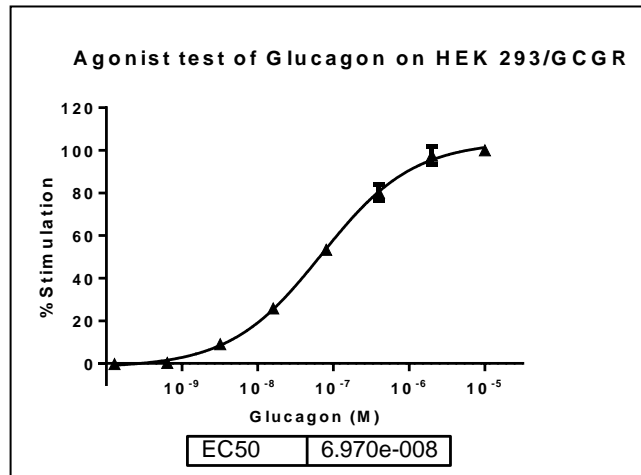
### Appendix

#### Appendix 1: Mycoplasma 160



**Agarose gel electrophoresis for Mycoplasma detection**

Lane M DNA Marker  
 Lane + 阳性质粒  
 Lane - lysis buffer (negative control)  
 Lane46 1-46 HEK 293/GCGR

**Appendix 2 : Calcium assay**


**Figure 2:** Glucagon-induced concentration-dependent stimulation of intracellular calcium mobilization in HEK 293/GCGR/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an GCGR receptor agonist, Glucagon. The intracellular calcium change was measured by FLIPR. The effects of agonist (%Stimulation) were plotted against the log of the cumulative doses (5-fold dilution) of Bradykinin (Mean ± SD, n = 3). The EC<sub>50</sub> of Glucagon on GCGR co-expressing with Gα15 in HEK293 cells was 69.7 nM. The S/B of Bradykinin on GCGR co-expressing with Gα15 in HEK293 cells was 35.

**Caution**

For research use only. Not intended for household use. If you have any questions about the Certificate of Analysis, please contact our customer service representative at 1-877-436-7274 (Toll-Free), or 1-732-885-9188.

Certified by:  Date: 02/27/2018  
 Department of Biologics Development Director