

APPENDIX

CELL LINE DESIGNATION
ORIGIN (PARENTAL CELL)
GENE INTRODUCED
RECEPTOR INTRODUCED:

Neuropeptide Y Receptor Y1 cell line (CB-80300-244)
HEK 293-CNG cell (CB-80200-200)
Genbank LocusID 4886
Human Neuropeptide Y Receptor Y1 (NCBI protein database
NP_000900.1)

USAGE

- cAMP assay for Gi-coupled human Neuropeptide Y Receptor Y1 (NPY1R).
- HEK293-CNG cells (CB-80200-200) without transfected Neuropeptide Y Receptor Y1 are used as a negative control.

QUALITY CONTROL

1. This cell line has been tested negative for *Mycoplasma sp.*
2. This cell line has been tested positive for Neuropeptide Y Receptor Y1 specific response.
3. Surviving rate: More than 2.5 million/vial on the second day after thawing.
4. The receptor specific activity is stable for 10 weeks continuous passage.

CELL CULTURE CONDITION

1. Growth medium: 90% DMEM, 10% FBS, 250 $\mu\text{g/ml}$ G418 and 1 $\mu\text{g/ml}$ puromycin
2. Freezing medium: 10% DMSO, 90% FBS

DATA EXAMPLE

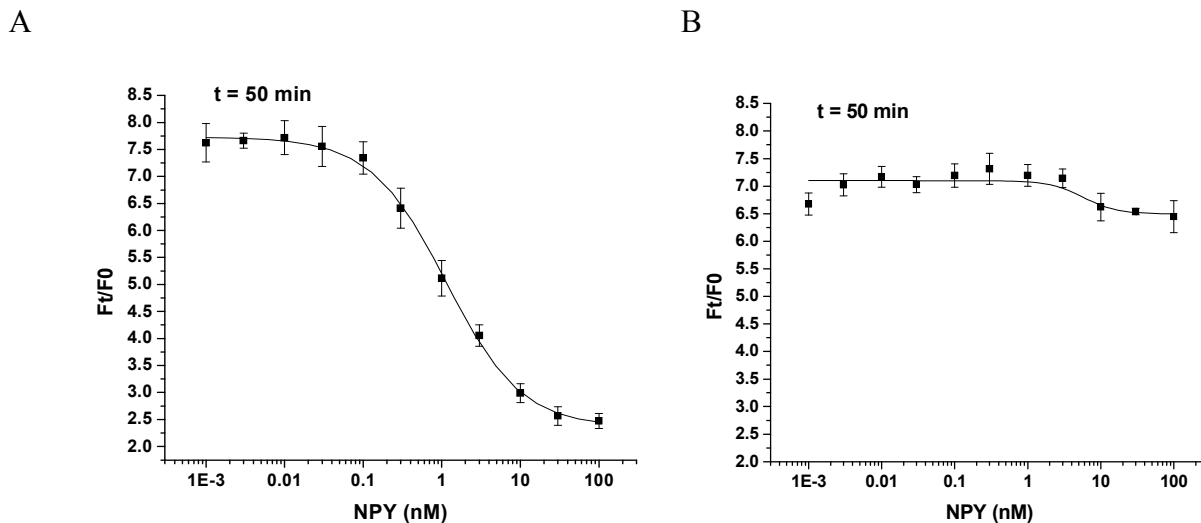


Figure 1. Response of ACTOne NPY1R cell line & parental cell line to NPY.

ACTOne Neuropeptide Y Receptor Y1 cells and parental cells (CB-80200-200) were plated overnight in 20 μl culture medium on a BD Biocoat 384 well plate. The next day, cells were dye-loaded with 20 $\mu\text{l/well}$ of 1X Dye-loading solution (ACTOne Membrane Potential Assay Kit). After 2 hours of incubation at room temperature, two readings were obtained prior to and 50 min after the addition of NPY. Ratios of the two readings (F/F0) are plotted in the figure.

A. Dose response curve of NPY in ACTOne Neuropeptide Y Receptor Y1 cell line. EC50 = 1.14 nM in the presence of PDE inhibitor Ro20-1724 and β -adrenoceptor agonist isoproterenol.

B. Parental cells do not respond to NPY.