

APPENDIX

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

Neuropeptide Y Receptor Y5 cell line (CB-80300-280)
HEK 293-CNG cell (CB-80200-200)
Genbank LocusID 4889
Human Neuropeptide Y Receptor Y5 (NCBI protein database EAX04840.1)

USAGE

- cAMP assay for Gi-coupled human Neuropeptide Y Receptor Y5.
- HEK293-CNG cells (CB-80200-200) without transfected NPY5R is 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 Y5 specific response.
3. Surviving rate: More than 2.5 million/vial on the second day after thawing.

CELL CULTURE CONDITION

1. Growth medium: 90% DMEM, 10% FBS, 250 µg/ml G418 and 1 µg/ml puromycin
2. Freezing medium: 10% DMSO, 90% FBS

DATA EXAMPLE

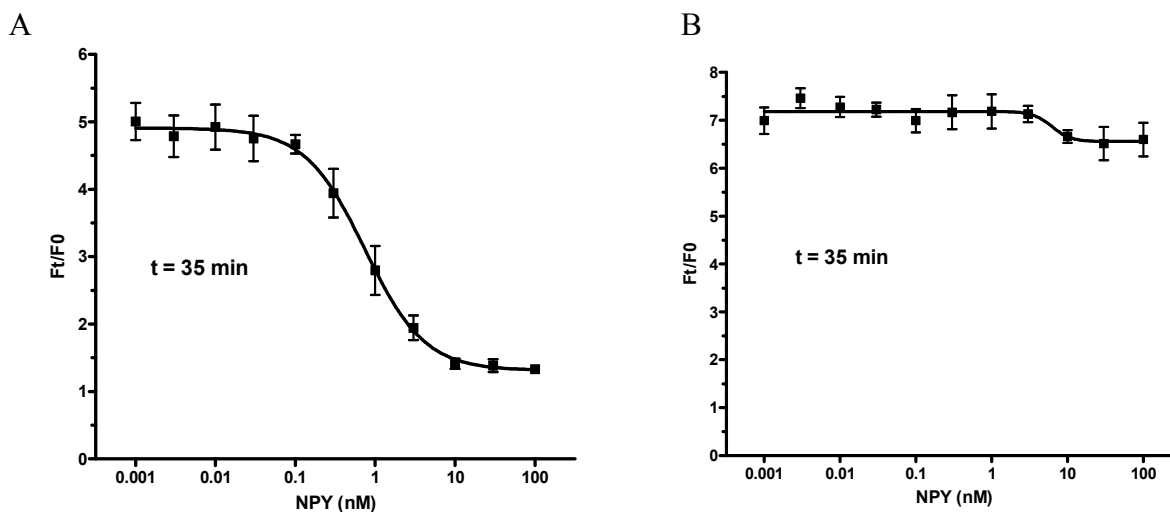


Figure 1. Response of ACTOne NPY5R cell line & parental cell line to NPY.

ACTOne NPY5R cells and parental cells (CB-80200-200) were plated overnight in 20 µl culture medium on a BD Bicoat 384 well plate. The next day, cells were dye-loaded with 20 µl/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/F_0) are plotted in the figure.

- A. Dose response curve of NPY in ACTOne NPY5R cell line. EC50 = 0.74 nM in the presence of PDE inhibitor Ro20-1724 and β -adrenoceptor agonist isoproterenol.**
- B. Parental cells do not respond to NPY.**