

## APPENDIX

### CELL LINE DESIGNATION

5-hydroxytryptamine (serotonin) receptor 7B cell line  
(CB-80200-240)

### ORIGIN (PARENTAL CELL)

HEK 293-CNG cell (CB-80200-200)

### GENE INTRODUCED

Genbank LocusID 3363

### RECEPTOR INTRODUCED:

Human 5-hydroxytryptamine (serotonin) receptor 7B (NCBI  
protein database NP\_062874.1)

### USAGE

- cAMP assay for Gs-coupled human 5-hydroxytryptamine (serotonin) receptor 7B (HTR7B).
- HEK293-CNG cells (CB-80200-200) without transfected 5-hydroxytryptamine (serotonin) receptor 7B are used as a negative control.

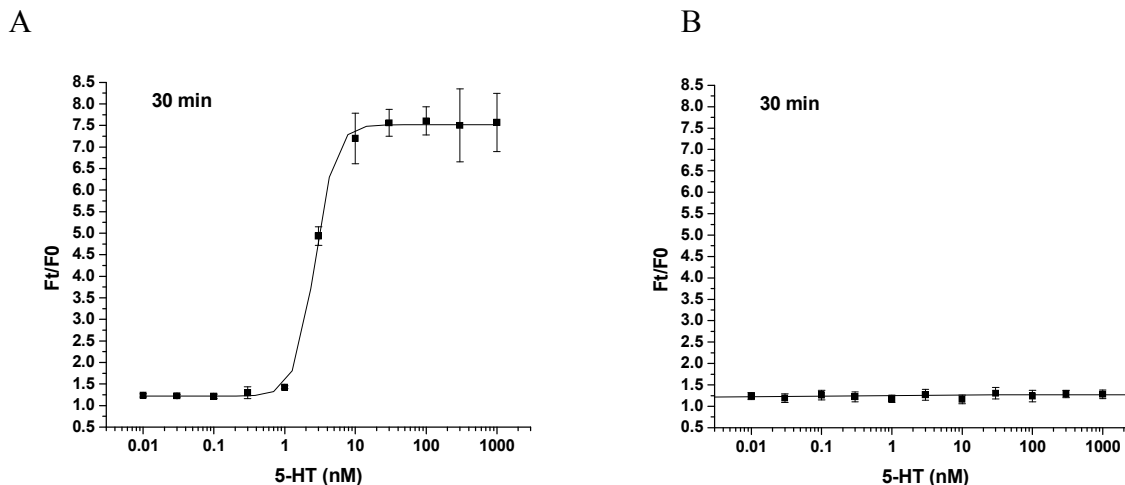
### QUALITY CONTROL

1. This cell line has been tested negative for *Mycoplasma sp.*
2. This cell line has been tested positive for 5-hydroxytryptamine (serotonin) receptor 7B 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 for maintaining the cells: 90% DMEM, 10% FBS, 250 µg/ml G418 and 1 µg/ml puromycin
2. Growth medium for ACTOne Assay: 90% DMEM, 10% FBS (CHARCOAL STRIPPED), 250 µg/ml G418 and 1 µg/ml puromycin
3. Freezing medium: 10% DMSO, 90% FBS

### DATA EXAMPLE



#### Figure 1. Response of ACTOne HTR7B cell line & parental cell line to 5-HT.

ACTOne 5-hydroxytryptamine (serotonin) receptor 7B 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 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 30 min after the addition of 5-HT. Ratios of the two readings (F/F<sub>0</sub>) are plotted in the figure.

- A. Dose response curve of 5-HT in ACTOne 5-hydroxytryptamine (serotonin) receptor 7B cell line. EC<sub>50</sub> = 28 nM in the presence of PDE inhibitor Ro20-1724.**
- B. Parental cells do not respond to 5-HT.**