

## human Serotonin 5-HT<sub>2A</sub> Receptor Cell Line

Product Number: ES-313-C

Lot Number: 3195544

### Material Provided

Cells: 2 x 1 mL frozen aliquot (ES-313-CV)  
Format: ~2.5 x 10<sup>6</sup> cells /mL in freezing medium

### Product Information

Cellular Background: CHO-K1

Cell Line Development: Our proprietary bicistronic expression plasmid containing the sequence coding for the human Serotonin 5-HT<sub>2A</sub> receptor was transfected in CHO-K1 cells. Geneticin-resistant clones were obtained by limit dilution and compared for receptor expression levels using a radioligand binding assay. The clone with the highest receptor expression level was selected for characterization in binding and functional assays.

DNA Sequence: Identical to coding sequence of GenBank NM\_000621.3.

Corresponding Protein Sequence: Identical to GenBank P28223.2.

Receptor expression level (B<sub>max</sub>): Estimated to be 4.1 ± 1.6 pmol/mg protein, using [<sup>3</sup>H]Ketanserin.

K<sub>d</sub> for the above radioligand: 0.78 ± 0.44 nM

Shipping Conditions: Shipped on dry ice. Please ensure dry ice is still present in the package upon receipt or contact customer support.

Storage Conditions: Store in liquid nitrogen (vapor phase) immediately upon receipt.



## Quality Control

The EC<sub>50</sub> for a reference agonist was determined in Calcium flux assay. A mycoplasma test was performed using MycoAlert® (Lonza) mycoplasma detection kit. We certify that these results meet our quality release criteria.

α-methyl-5-HT (EC<sub>50</sub>):

N/A

Stability:

Cells were kept in continuous culture for at least 60 days and showed no decrease of receptor expression level in a saturation binding assay (stable B<sub>max</sub> and K<sub>d</sub>) and no decrease in functional response (EC<sub>50</sub>, E<sub>max</sub> in calcium flux assay).

Mycoplasma:

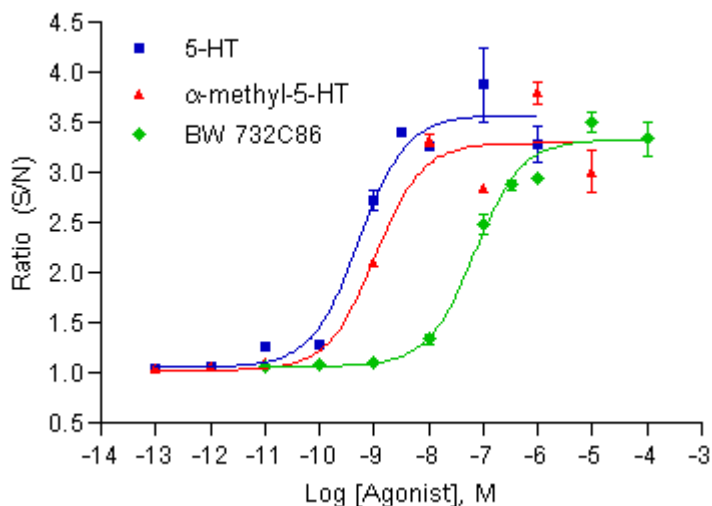
This cell line tested negative for mycoplasma.

## Assay Procedures

We have shown for many of our GPCR cell lines that freshly thawed cells respond with the same pharmacology as cultured cells. All of our products validated in this way are available as frozen ready-to-use cells in our catalogue. This demonstrates that cells can be prepared and frozen in advance of a screening campaign simplifying assay logistics.



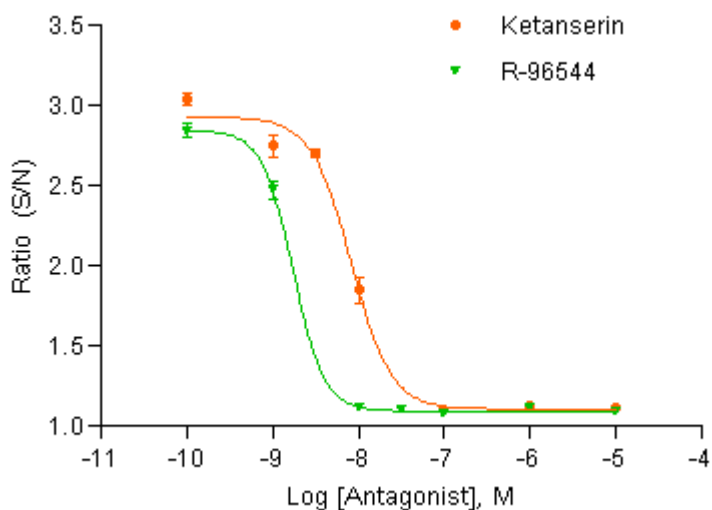
Typical Product Data - Calcium Assay (Fluorescence)



Agonist	EC <sub>50</sub> (M)
α-methyl-5HT	1.0 x 10 <sup>-9</sup>
5-HT	5.3 x 10 <sup>-10</sup>
BW 732C86	7.0 x 10 <sup>-8</sup>

Figure1. Agonist Response in Fluo-4 Calcium Flux assay

An agonist dose-response experiment was performed in 96-well format using 25000 cells/well. Fluo-4. Fluorescence was measured on a FDSS 6000 instrument (Hamamatsu Photonics). Data from a representative experiment are shown.



Antagonist	IC <sub>50</sub> (M)
Ketanserin	8.2 x 10 <sup>-9</sup>
R-96544	1.7 x 10 <sup>-9</sup>

Figure 2. Antagonist Response in Fluo-4 Calcium Flux assay

An antagonist dose-response experiment was performed in 96-well format using 25000 cells/well. 5-HT was used as reference agonist, at a final concentration of 2.4 nM (EC<sub>80</sub>). Fluorescence was measured on a FDSS 6000 instrument (Hamamatsu Photonics). Data from a representative experiment are shown.

Typical Product Data -Radioligand Binding Assay (Filtration)

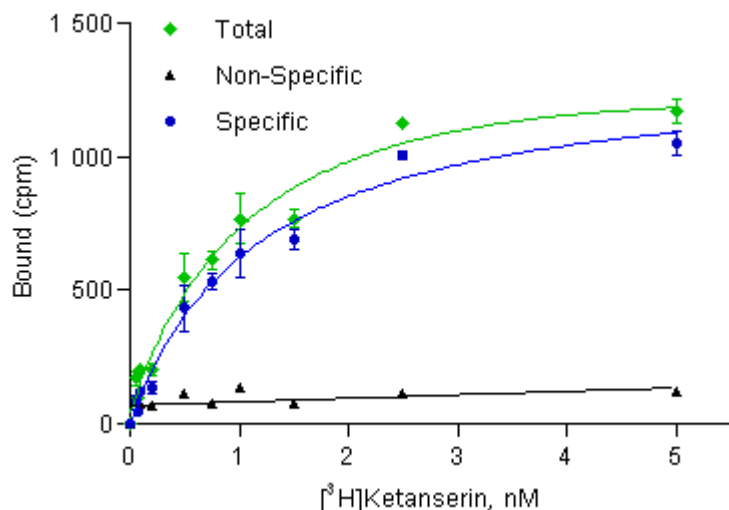
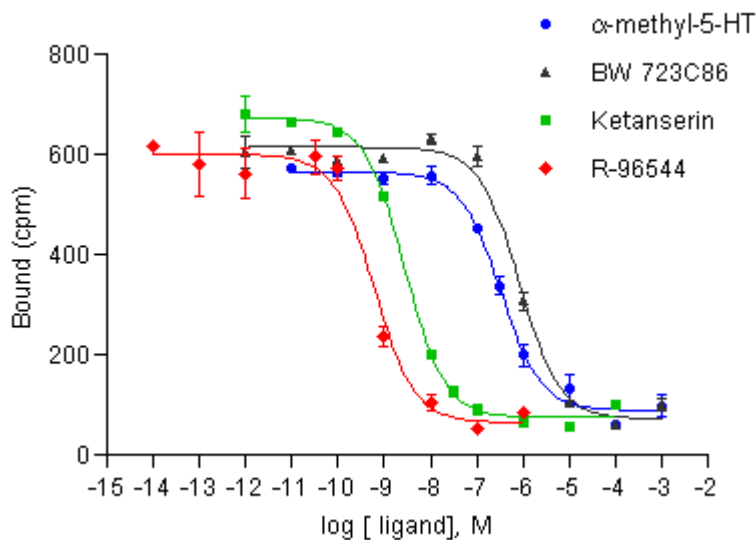


Figure 3: Saturation Binding Assay Curve (Filtration)

A saturation binding assay was performed in 96-well format using 5 µg membranes/well. Counts per minute (cpm) were measured on a TopCount® instrument. Data from a representative experiment are shown.



Agonist / Antagonist	IC <sub>50</sub> (M)
<b>α-methyl-5-HT</b>	<b>3.3 x 10<sup>-7</sup></b>
<b>BW 723C86</b>	<b>8.4 x 10<sup>-7</sup></b>
<b>Ketanserin</b>	<b>2.7 x 10<sup>-9</sup></b>
<b>R-96544</b>	<b>6.1 x 10<sup>-10</sup></b>

Figure 4: Competition Binding Assay Curve (Filtration)

A competition binding assay was performed in 96-well format using 5 µg membranes/well. Displacement of 1 nM [3H]-Ketanserin was used. Counts per minute (cpm) were measured on a TopCount® instrument. Data from a representative experiment are shown.



## Calcium Assay Procedure (Fluorescence)

Dye solution: 5  $\mu$ M Fluo-4 AM (Molecular Probes, P-6867), 1 mg/mL Pluronic acid in Assay Buffer

Assay Buffer: 2.5 mM Probenicid, 0.1% BSA, 0.05% Gelatin, 135 mM NaCl, 5 mM KCl, 1.8 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 10 mM HEPES, 5.6 mM Glucose, pH 7.4

Controls: Maximal Signal: 0.4% Triton X-100 (0.2% final) in Assay Buffer

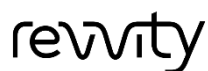
Minimum signal: 0.4% Triton X-100 (0.2% final), 20 mM EGTA (10 mM final) in Assay Buffer

Reader: FDSS 6000 (Hamamatsu Photonics), Excitation 480 nm / Emission 520 to 560 nm, 96-well

Day 1			
1. Cell Culture and Harvesting:	Grow cells (mid-log phase) in culture medium without antibiotics for 18 hours, Detach gently with PBS/0.5 mM EDTA, pH 7.4, Recover by centrifugation, Resuspend in medium without antibiotics at $2.5 \times 10^5$ cells/mL.		
2. Cell Seeding	Distribute 100 $\mu$ L (i.e. 25,000 cells) in each well of a 96 well black, clear bottom TC sterile plate, incubate overnight in a cell culture incubator (37°C, 5% $\text{CO}_2$ ).		
Day 2			
3. Cell Loading	Remove the media and add 100 $\mu$ L/well of Dye solution.		
4. Incubation	Incubate the assay plate for 1 hour at 37°C in a cell culture incubator.		
5. Ligands and compound plates preparation:	Prepare serial dilutions of 2x concentrated ligands in Assay Buffer, Dispense 100 $\mu$ L/well of diluted ligand in a 96-well plate. <i>Note: Assay can be miniaturized to 384-well format.</i>		
6. Dye Washing	Drain the media and wash the wells twice with 100 $\mu$ L/well Assay Buffer,		
7. Buffer/Antagonist addition	<table border="1" style="width: 100%;"> <tr> <td style="width: 50%;">Agonist assay: Add Assay Buffer to make a total of 50 <math>\mu</math>L</td> <td style="width: 50%;">Antagonist Assay: Add 2x antagonist dilution in Assay Buffer to make a total of 50 <math>\mu</math>L</td> </tr> </table>	Agonist assay: Add Assay Buffer to make a total of 50 $\mu$ L	Antagonist Assay: Add 2x antagonist dilution in Assay Buffer to make a total of 50 $\mu$ L
Agonist assay: Add Assay Buffer to make a total of 50 $\mu$ L	Antagonist Assay: Add 2x antagonist dilution in Assay Buffer to make a total of 50 $\mu$ L		
8. Equilibration	Incubate the plate for 20 min at room temperature in the dark.		
9. Plate Reading:	<table border="1" style="width: 100%;"> <tr> <td style="width: 50%;">Using the reader's injection system, inject 50 <math>\mu</math>L per well of 2x agonist solutions in Assay Buffer, and immediately record relative light emission for 90 seconds.</td> <td style="width: 50%;">Using the reader's injection system, inject 50 <math>\mu</math>L per well of 2x concentrated reference agonist in Assay Buffer (final <math>\text{EC}_{80}</math> concentration), and immediately record relative light emission for 90 seconds.</td> </tr> </table>	Using the reader's injection system, inject 50 $\mu$ L per well of 2x agonist solutions in Assay Buffer, and immediately record relative light emission for 90 seconds.	Using the reader's injection system, inject 50 $\mu$ L per well of 2x concentrated reference agonist in Assay Buffer (final $\text{EC}_{80}$ concentration), and immediately record relative light emission for 90 seconds.
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10. Data Analysis:	The fluorescent signal is expressed as the ratio relative to the first measurement (i.e. before dispensing), and the maximal value of this ratio during the measurement interval is used to draw sigmoidal dose-response curves.		

### Important Notes:

- Probenicid is prepared as a 250 mM solution in a 50:50 mixture of 1N NaOH : Assay Buffer.



## Membrane Radioligand Binding Assay Procedure (Filtration)

Note: The following are recommended assay conditions and may differ from the conditions used to generate the typical data shown in the above section.

Assay Buffer: 50 mM Tris pH 7.4, 0.1% ascorbic acid, 4 mM CaCl<sub>2</sub>

Wash Buffer: 50 mM Tris-HCl pH 7.4

Radioligand: [<sup>3</sup>H]-Ketanserin (Revvity # NET791)

Filters: Unifilter 96 GF/C (Revvity # 6055690)

### Membrane Binding Protocol:

Binding assays were performed in 550 µL total volume according to the following conditions. All dilutions are performed in assay buffer:

1. Membrane dilution:	5 µg of membranes per well, diluted in order to dispense 500µL/well. Keep on ice.
2. Assembly on ice (in 96 Deep well plate)  Saturation Binding:	<ul style="list-style-type: none"><li>• 25 µL of assay buffer or of unlabeled ligand (Mianserin, 1 µM final) for determination of non specific binding</li><li>• 25 µL of radioligand at increasing concentrations (see figure 3)</li><li>• 500 µL of diluted membranes</li></ul>
Competition Binding:	<ul style="list-style-type: none"><li>• 25 µL competitor ligand at increasing concentrations (see figure 4)</li><li>• 25 µL of radioligand (1 nM final)</li><li>• 500 µL of diluted membranes</li></ul>
3. Incubation:	60 min at 27°C.
4. Filters preparation:	GF/C filters were presoaked in 0.5 % PEI at room temperature for at least 30 min.
5. Filtration:	Aspirate and wash 9 x 500 µL with ice cold wash buffer using a FilterMate Harvester.
6. Counting:	Add 30 µL/well of MicroScint™-O (Revvity # 6013611), cover filter with a TopSeal-A (Revvity # 6050185) and read on a TopCount®.

## References

1. Saltzman A.G., Morse B, Whitman MM, Ivanshchenko Y, Jaye M, Felder S. (1991) Cloning of the human serotonin 5-HT<sub>2</sub> and 5-HT<sub>1C</sub> receptor subtypes. *BBRC* 181:1469-1478
2. Hoyer D, Schoeffter P (1991) 5-HT receptors: subtypes and second messengers. *Recept Res.*11:197-214.
3. Zifa E, Fillion G (1992) 5-Hydroxytryptamine receptors. *Pharmacol Rev.* 44:401-458.
4. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev.* 46:157-203.
5. Kaumann AJ, Levy FO (2006) 5-hydroxytryptamine receptors in the human cardiovascular system. *Pharmacol Ther.* 111(3):674-706.
6. Filip M, Bader M (2009) Overview on 5-HT receptors and their role in physiology and pathology of the central nervous system. *Pharmacol Rep.* (5):761-77.





## Materials and Instrumentation

The following tables provide the references of compounds and reagents used or recommended for the characterization of the human Serotonin 5-HT<sub>2A</sub> receptor ValiScreen<sup>®</sup> cell line, as well as some advice on how to use these compounds:

Table 1. References of compounds used for functional characterization and binding assays

Name	Provider	Cat no	Working Stock Solution
$\alpha$ -methyl-5-HT ( $\alpha$ -Me-serotonin)	Sigma	M110	1 mM in dH <sub>2</sub> O - prepare fresh
5-Hydroxytryptamine (5-HT)	Sigma	H-9523	10 mM in dH <sub>2</sub> O - prepare fresh
Ketanserin	Tocris	0908	10 mM in dH <sub>2</sub> O
Mianserin	Sigma	M2525	10 mM in Ethanol
BW 723C86	Tocris	1059	10 mM in DMSO
R-96544	Tocris	1742	100 mM in dH <sub>2</sub> O
[ <sup>3</sup> H]-Ketanserin	Revvity	NET791	N/A

Table 2. References of cell culture media and assay buffers

Name	Provider	Cat no
HAM's F-12	Hyclone	SH30026.02
DMEM	Hyclone	SH30022.02
Advanced DMEM/F12 (serotonin receptors)	Invitrogen	12634-010
EMEM	BioWitthaker	06-174G
EX-CELL DHFR <sup>-</sup> media (DHFR deficient cell lines)	Sigma	C8862
FBS	Wisent	80150
FBS dialyzed	Wisent	80950
G418 (geneticin)	Wisent	400-130-IG
Zeocin	Invitrogen	R25005
Blasticidin	Invitrogen	R210-01
Puromycin	Wisent	400-160-EM
Standard HBSS (with CaCl <sub>2</sub> and MgCl <sub>2</sub> )	GIBCO	14025
HEPES	MP Biomedicals, LLC	101926
BSA, Protease-free	Sigma	A-3059
PEI	Sigma	P3143
Trypsin-EDTA	Hyclone	SH30236.02
Sodium Pyruvate	GIBCO	11360
L-Glutamine	GIBCO	25030
NEAA (non-essential amino acids)	GIBCO	11140
Forskolin	Sigma	F6886

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Revvity  
940 Winter Street  
Waltham, MA 02451 USA

(800) 762-4000  
www.revvity.com

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