human Serotonin Transporter Receptor Cell Line

Product No.: RBHSTM-K

Lot No.: 2651523

Material Provided

Cells:	2 x 1 mL frozen aliquot (RBHSTM-WV)
Format:	~5 x 10^6 cells/mL in freezing medium

Product Information

Cellular Background:	HEK293
GenBank Accession Number:	NM_001045
Cell Line Development:	Our expression plasmid containing the sequence coding for the human Serotonin transporter receptor was transfected in HEK293 cells. Geneticin-resistant clones were obtained by limit dilution and compared for receptor expression levels by radioligand binding assay. The clone with the highest receptor expression level was selected for characterization in binding and functional assays.
Receptor expression level (B_{max}):	Estimated to be 11 pmol/mg protein, using [^{3}H] Imipramine.
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

Mycoplasma:	This cell line tested negative for mycoplasma.

Recommended Cell Culture Conditions (HEK-293)

- The recommended media catalogue number and supplier reference information are listed in this Product Technical Data Sheet (last page). Media composition is specifically defined for each cell type and receptor expression selection. The use of incorrect media or component substitutions can lead to reduced cell viability, growth issues and/or altered receptor expression.
- Cells undergo major stress upon thawing, and need to adapt to their new environment which may initially affect cell adherence and growth rates. The initial recovery of the cells, and initial doubling time, will vary from laboratory to laboratory, reflecting differences in the origin of culture media and serum, and differences in methodology used within each laboratory.
- For the initial period of cell growth (i.e. until cells have reached Log-phase, typically 4-10 days), we strongly recommend removal of the antibiotics (G418, Zeocin™, Puromycin, Blasticidin, Hygromycin, Penicillin and Streptomycin) from the culture media. Immediately after thawing, cells may be more permeable to antibiotics, and a higher intracellular antibiotic concentration may result as a consequence. Antibiotics should be re-introduced when cells have recovered from the thawing stress.

Growth Medium:	DMEM, 10% FBS dialyzed, 1mM Sodium pyruvate, 100 µg/ml G418 (receptor expression selection).
Freezing Medium:	DMEM, 10% FBS dialyzed, 1mM Sodium pyruvate with 10% DMSO, without selection agents.

Thawing Cells: Using appropriate personal protective equipment, rapidly place the frozen aliquot in a 37°C water bath (do not submerge) and agitate until its content is thawed completely. Immediately remove from water bath, spray aliquot with 70% ethanol and wipe excess. Under aseptic conditions using a sterile pipette, transfer content to a sterile centrifuge tube containing 10 mL growth medium without antibiotics, pre-warmed at 37°C, and centrifuge (150 x g, 5 min). Discard supernatant using a sterile pipette. Resuspend cell pellet in 10 mL of pre-warmed growth medium without antibiotics by pipetting up and down to break up any clumps, and transfer to an appropriate culture flask (e.g. T-25, T-75 or T-175, see recommended seeding density below). Cells are cultured as a monolayer at 37°C in a humidified atmosphere with 5% CO_2.

Recommended Seeding Density:	Thawing:	60,000 – 65,000 cells/cm^2
	Log-phase:	41,000 – 45,000 cells/cm^2

Troubleshooting: Initial doubling time can vary between 18 and 96 hours (Average = 25 hours). If cells are still not adhering after 48 hours or grow very slowly, we recommend maintaining the cells in culture and not replacing the media before 5-6 days (cells secrete factors that can help with adherence and growth). If confluence is still <50% after 5-6 days, it is recommended that you replace the media with fresh media (without antibiotics). Do not passage the cells until they reach 80-90% confluence (Log-phase). If cells have not recovered after 10-12 days, please contact our Technical Support.

Culture Protocol: Under aseptic conditions, cells are grown to 80% confluence (Log-phase) and trypsinized (0.05% trypsin / 0.5 mM EDTA in calcium and magnesium-free PBS). See recommended seeding density for Log-phase above.

Banking Protocol: Cells are grown to 70-80% confluence (Log-phase). Under aseptic conditions, remove medium and rinse the flask with an appropriate volume of calcium and magnesium-free PBS (example 10 mL for T-175). Trypsinize (0.05% trypsin / 0.5 mM EDTA in calcium and magnesium-free PBS) to detach cells (example 5 mL for T-175), let stand 5-10 min at 37°C. Add fresh, room temperature growth medium (without antibiotics) to stop trypsinization and dilute EDTA (example 10 mL for T-175). Transfer cells to a sterile centrifuge tube and centrifuge (150 x g, 5 min). Discard supernatant using a sterile pipette. Resuspend cell pellet in ice-cold freezing medium by pipetting up and down to break up any clumps. Count cells and rapidly aliquot at the selected cell density (e.g. 2.5 x 10^6 cells/mL) in sterile polypropylene cryovials. Use appropriate material to ensure slow cooling (about -1°C/min) until -70°C. Transfer vials into a liquid nitrogen tank (vapor phase) for storage.

Historical Cell Line Validation using Membrane Preparation*

Saturation and Competition Binding Assay

Assay Buffer:	50 mM Tris-HCl pH 7.4, 120 mM NaCl, 5 mM KCl
Wash Buffer:	50 mM Tris-HCl pH 7.4, 154 mM NaCl
Binding Protocol:	Binding assays are performed in 550 µL total volume according to the following conditions:
1 - Membrane dilution:	0.05 mL of membranes + 24.95 mL assay buffer (1:500 dilution)
2 - Incubation:	25 µL of incubation buffer or Imipramine (Sigma I0899) 200 µM final for non specific binding (Saturation binding assay) *For competition binding assay: 25 µL of reference compounds at decreasing concentrations (see figure 2)* 25 µL of radioligand at the appropriate concentration (see graph below) 500 µL of diluted membranes
3 - Incubation time:	30 minutes at 27°C
4 - Filtration:	aspirate and wash 9 x 500 µL with ice cold wash buffer over GF/C filter (presoaked in 0.5 % PEI).

* *Membrane preparation available as RBHSTM400UA*

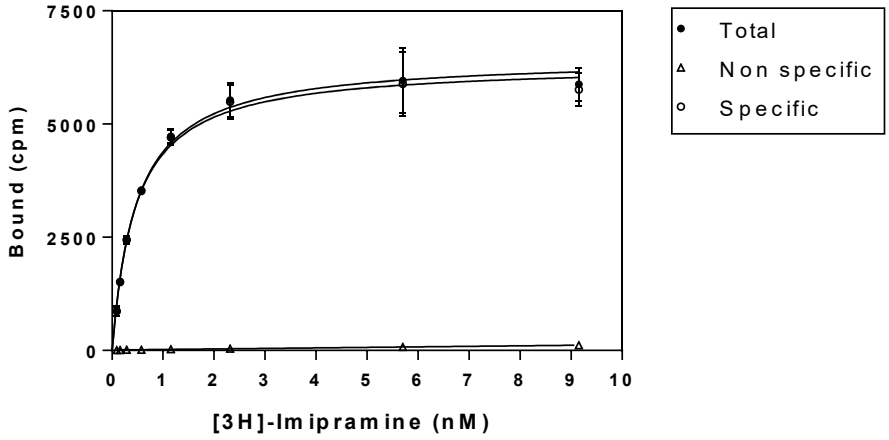

Figure 1: Saturation binding assay curve (filtration)
96-well saturation binding assay curve (9 µg membranes/well, TopCount®) using [^{3}H]-Imipramine (Revvity NET576 Lot No.: 1964403).

Typical Product Data

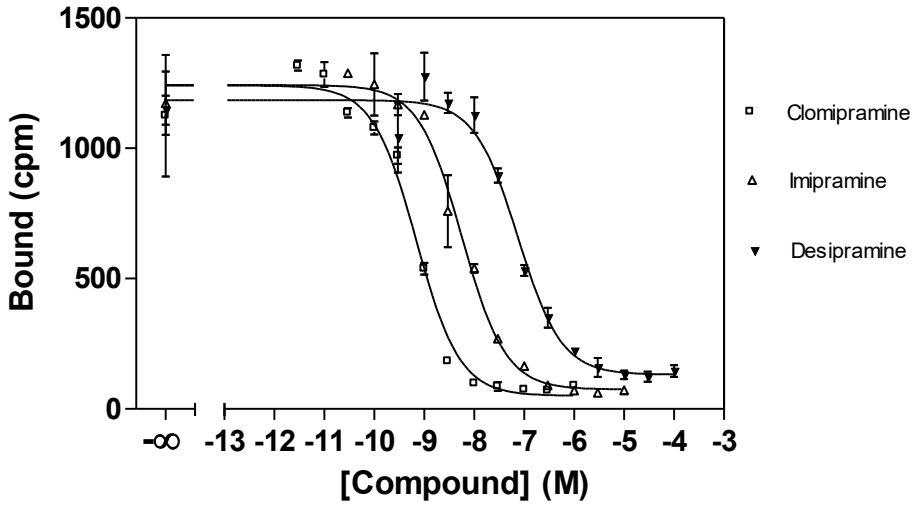

Figure 2: Competition binding assay curve (filtration)
96-well competition binding assay curve (9 µg membranes/well,TopCount®).Recommended radioligand concentration = 2 nM.

*Even though two sites can be observed occasionally with some ligands, the data presented is derived from single site fitting.

Reference Compounds	Ki (nM)
Clomipramine	0.53
Imipramine	4.4
Desipramine	57

Reference of Cell Culture Media

Name	Provider	Cat. Number
Ham's Nutrient Mixture F-12	Fisher Scientific	SH30026.02
DMEM/High Glucose	Fisher Scientific	SH30022.02
Advanced DMEM/F12	ThermoFisher (Gibco)	12634-010
EMEM	Lonza	06-174G
EX-CELL® CHO DHFR-	Sigma	C8862
FBS	RMBIO	FBS-BBT
FBS dialyzed	Wisent	080950
G418 Sulfate	Wisent	400-130-IG
Zeocin™	ThermoFisher (Gibco)	R25005
Blasticidin	ThermoFisher (Gibco)	R210-01
Puromycin	Wisent	400-160-EM
Trypsin-EDTA	Fisher Scientific	SH30236.02
Sodium Pyruvate	ThermoFisher (Gibco)	11360
L-Glutamine	ThermoFisher (Gibco)	25030

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