Research use only. Not for use in diagnostic procedures.

ValiScreen® GPCR Cell Line

human Opioid delta Receptor Cell Line

Product No.: 6110549-K

Lot No.: 2533369

Material Provided

Cells: 2 x 1 mL frozen aliquot (6110549-WV)

Format: ~5 x 10⁶ cells/mL in freezing medium

Product Information

Cellular Background: HEK293

GenBank Accession Number: NM_000911 (C27F, A370R)

Cell Line Development: Our expression plasmid containing the sequence coding for the

human Opioid delta receptor was transfected in HEK293 cells. 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 [${}^{3}H$]-Naltrindole.

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 me
- This cell line does not use any antibiotics. Antibiotics are not permitted in the growth or freezing medium during the culture of these cells.

Growth Medium: MEM, 10% Fetal Bovine serum, (Heat Inactivated), 2mM Glutamine

Freezing Medium: MEM, 10% Fetal Bovine serum, (Heat Inactivated), 2mM Glutamine 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 prewarmed 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% CO₂.

Recommended Seeding Density: Thawing: 60,000 – 65,000 cells/cm²

Log-phase: 41,000 - 45,000 cells/cm²

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. 2.5×10^6 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, 10 mM MgCl₂, 1 mM EDTA

Wash Buffer: 50 mM Tris-HCl pH 7.4

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 Naltrindole (Sigma N115) 1 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: 60 minutes at 27 °C

4 - Filtration: aspirate and wash 9 x 500 μL with ice cold wash buffer over GF/C filter

(presoaked in 0.3% PEI).

^{*}Membrane preparation available as 6110549400UA

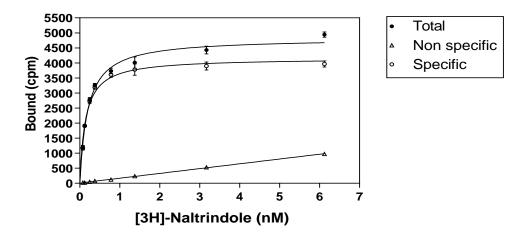


Figure 1: Saturation binding assay curve (filtration) 96-well saturation binding assay curve (9 µg membranes/well, TopCount®) using [³H]-Naltrindole (Revvity NET1065 Lot No.: CUSM03012018JB)



Typical Product Data

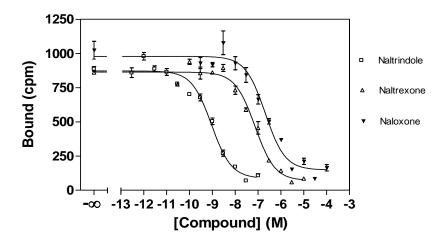


Figure 2: Competition binding assay curve (filtration) 96-well competition binding assay curve (9 µg membranes/well, TopCount®). Recommended radioligand concentration = 0.15 nM.

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

| Reference Compounds | Ki |
|---------------------|------|
| | (nM) |
| Naltrindole | 0.37 |
| Naltrexone | 30 |
| Naloxone | 83 |



Reference of Cell Culture Media

| Name | Provider | Cat. Number |
|--------------------------------------------------------------------------|----------------------|-------------|
| MEM/Earles | Hyclone | SH30024.02 |
| Advanced DMEM/F12 | ThermoFisher (Gibco) | 12634-010 |
| EMEM | Lonza | 06-174G |
| EX-CELL® CHO DHFR- | Sigma | C8862 |
| Fetal Clone II | Fisher Hyclone | SH30066.03 |
| FBS | Wisent | 80150 |
| FBS (Heat inactivated- fully thawed serum heated to 57'C for 30 minutes) | Wisent | 80150 |
| G418 Sulfate | Wisent | 400-130-IG |
| Zeocin™ | ThermoFisher (Gibco) | R25005 |
| Blasticidin | ThermoFisher (Gibco) | R210-01 |
| Puromycin | Wisent | 400-160-EM |
| Hygromycin D | ThermoFisher | |
| | Scientific | 10687010 |
| Trypsin-EDTA | Fisher Scientific | SH30236.02 |
| Sodium Pyruvate | ThermoFisher (Gibco) | 11360 |
| L-Glutamine | ThermoFisher (Gibco) | 25030 |

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