

Protocol of HTR2A-CHO Cell-based Assay for High-throughput Screening

DOCUMENT: *HTR2A-CHO_TOX21_SLP*

TITLE: Protocol of *HTR2A-CHO* cell-based assay for high-throughput screening.

ASSAY REFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
5-hydroxytryptamine receptor 2A (HTR2A)	CHO	Hamster	Ovary	Fluorescence	Codex	Gq-coupled HTR2A signaling

QUALITY CONTROL PRECAUTIONS:

1. Cells should be grown to reach 80 to 90% confluence.
- 2.

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Vender	Catalog Number
DMEM/F12	Sigma	D8437
FBS, charcoal stripped	Sigma	F6757
Penicillin/Streptomycin	Invitrogen	15140
DPBS	Invitrogen	14190
0.05% Trypsin/EDTA	Invitrogen	25300
Puromycin	Invitrogen	A11138
Recovery™ Cell Culture Freezing Medium	Invitrogen	12648
DOI	Sigma	D101
Ketanserin	Sigma	S006
Cal-520 calcium assay kit	AAT Bioquest	36318
Black-clear bottom, 1536-well assay plates	Greiner Bio-One	
BioRAPTR™, Microfluidic Workstation	Beckmen	
FDSS 7000EX kinetic plate reader	Hamamatsu	

PROCEDURE:

1. Cell handling:
 - 1.1. Medium Required

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
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DMEM	90%	90%	90%	-
FBS	10%	10%	10%	-
Penicillin-Streptomycin	100 U/mL-100 µg/mL	100 U/mL-100 µg/mL	100 U/mL-100 µg/mL	-
Puromycin	10 µg/mL	-	-	-
Recovery™ Cell Culture Freezing Medium	-	-	-	100%

1.2. Thawing method

- 1.21 Place 14 mL of pre-warmed thaw medium into a 15 mL of conical tub.
- 1.22 Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes.
- 1.23 Decontaminate the vial by wiping with 70% ethanol before opening in a biological safety cabinet.
- 1.24 Transfer the vial contents drop-wise into 14 mL of thaw medium in a sterile 15-mL conical tub.
- 1.25 Centrifuge cells at 900 rpm for 4 minutes and resuspend in thaw medium.
- 1.26 Transfer contents to the T75 tissue culture flask containing Thaw Medium and place flask in a humidified 37°C/5% CO2 incubator.
- 1.27 Switch to growth medium at first passage.

1.3. Propagation method

- 1.31 Aspirate medium, rinse once in DPBS, add 0.05% Trypsin/EDTA and swirl to coat the cell evenly.
- 1.32 Add an equal volume of Growth Medium to inactivate Trypsin after 2-3 minutes incubation at 37°C.
- 1.33 Centrifuge cells at 900 rpm for 4 minutes and resuspend in growth medium.
- 1.34 Cell should be passage or fed at least twice a week.

2. Assay protocol for antagonist mode

- 2.1 Harvest cells and resuspend in assay medium.
- 2.2 Dispense 2000cells/3µL/well into 1536-well tissue treated black-clear-bottom plates using a Multi-drop dispenser.
- 2.3 After the cells were incubated at 37°C for 18 hours, add 3µl of Loading Dye to each well.
- 2.4 Incubate the plates for 1 h at 37°C.
- 2.5 Incubate the plates for 15 m at room temperature
- 2.6 Add 23 nL of compounds dissolved in DMSO, positive controls or DMSO by a PinTool.
- 2.7 Read the fluorescence intensity in FDSS 7000EX kinetic plate reader with a filter set of Ex/Em=480/540 for 3 min at 1 sec intervals.
- 2.8 For antagonist mode, incubate the plates for 5 m at room temperature after step 2.6
- 2.9 Add 1.5 µL of Acetylcholine at 4 nM (final conc.) to each well
- 2.10 Read the fluorescence intensity in FDSS 7000EX kinetic plate reader with a filter set of Ex/Em=480/540 for 2 min at 1 sec intervals

3. Assay Performance

HTR2A-CHO agonist	Online Validation (Mean ± SD)
EC50 (nM)	< 1pm
S/B	5.09 ± 0.61
CV (%)	14.73 ± 2.88
Z'	0.60± 0.08

HTR2A-CHO antagonist	Online Validation* (Mean ± SD)
EC50 (nM)	Not available

S/B	7.23 ± 1.11
CV (%)	5.91 ± 1.26
Z'	0.77 ± 0.09

* Used the data of DMSO with and without DOI