

Protocol of DRD2-Hek293 Cell-based Assay for High-throughput Screening

DOCUMENT: *DRD2-Hek293_TOX21_SLP*

TITLE: Protocol of *DRD2-Hek293* cell-based assay for high-throughput screening.

ASSAY REFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
D2 dopamine receptor (DRD2)	Hek293	Human	Embryonic kidney cells	Fluorescence	Codex	Gi-coupled DRD2 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	Invitrogen	10995
Fetal bovine serum	Invitrogen	26140
Penicillin/Streptomycin	Invitrogen	15140
DPBS	Invitrogen	14190
0.05% Trypsin/EDTA	Invitrogen	25300
Puromycin	Invitrogen	A11138
G418	Invitrogen	10131035
Recovery™ Cell Culture Freezing Medium	Invitrogen	12648
Haloperidol	Sigma	H1512
Quinpirole	Sigma	Q102
Ro20-1724	Sigma	B8279
NHK477	Sigma	N3290-5MG
cAMP Gi kit	Cisbio	62AM9PEC
White-solid bottom, 1536-well assay plates	Greiner Bio-One	Greiner, 789093
PinTool	Kalypsys	
BioRAPTR™, Microfluidic Workstation	Beckmen	
EnVision plate reader	Perkin Elmer	

PROCEDURE:

1. Cell handling:
 - 1.1. Medium Required

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
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	1 µg/mL	-	-	-
G418	250 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 600cells/3µL/well into 1536-well tissue treated white-solid-bottom plates using a Multi-drop dispenser.
- 2.3 After the cells were incubated at 37°C for 24 hours, add 1 µL of Ro 20-1724 (final concentration is 0.1 mM) to each well. Following by adding 23 nL of compounds dissolved in DMSO, positive controls or DMSO by a PinTool.
- 2.4 Incubate the plates for 10 m at room temperature
- 2.5 Add 1 µL of mixture of NHK477 at 200nM and quinpirole at 12nM to each well.
- 2.6 Incubate the plates for 30 m at room temperature
- 2.7 Add 2.5 µL of cAMP-d2, and then add 2.5 µL of anti cAMP-Cryptate to each well using a BioRAPTR dispenser.
- 2.8 After one hour incubation at room temperature, measure fluorescence intensity at 665 and 620 nm emission and 340 nm excitation by an Envision detector. Data is expressed as the ratio of 665nm/620nm emissions.

3. Assay Performance

DRD2-HEK293-cAMP Antagonist	Online Validation (Mean ± SD)
EC50 (nM)	0.48±0.11
S/B	3.66 ± 0.16
CV (%)	13.1 ± 2.49
Z'	0.52 ± 0.11