**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 RFERENCES:**

| 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 |

**QALITY 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 |
| 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 NHK477 at final concentration of 200nM 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 agonist | Online Validation (Mean ± SD) |
|---|---|
| EC50 (nM) | 3.43±1.50 |
| S/B | 4.84 ± 0.37 |
| CV (%) | 9.33 ± 1.62 |
| Z’ | 0.76± 0.03 |