

Protocol of Kiss1R_ HEK293 Cell-based Assay for High-throughput Screening

DOCUMENT: Kiss1R_TOX21_SLP_Version1.0
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ASSAY REFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Kiss1R (Recombinant)	HEK293	Human	Embryonic kidney	Fluorescence intensity	Jimmy Lu	G-protein-coupled receptor

QUALITY CONTROL PRECAUTIONS:

1. Maintain cells below 85-90% confluence
2. Feed or passage cells twice a week

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM	ThermoFisher Scientific	ThermoFisher Scientific / 11995065
Fetal Bovine Serum	Hyclone	Hyclone / SH30071.03
Penicillin/Streptomycin	ThermoFisher Scientific	ThermoFisher Scientific / 15140122
Puromycin Dihydrochloride	ThermoFisher Scientific	ThermoFisher Scientific / A1113803
Recovery Cell culture Freezing Medium	Invitrogen	Invitrogen / 12648
0.05% Trypsin-EDTA	Invitrogen	Invitrogen / 25300
1536-well black clear plates	Greiner Bio-One	Greiner Bio-One / 789092-F
MULTIDROP COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD	Beckman Coulter	Beckman Coulter
FDSS 7000EX Plate Reader	Hamamatsu	Hamamatsu/7000EX

Calcium Assay Kit	AAT Bioquest	AAT Bioquest / 36319
Kisspeptin 10 (Agonist control compound)	Tocris	Tocris / 2570
Kisspeptin 234 (Antagonist control compound)	Tocris	Tocris / 3881

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
DMEM	90%	90%	90%	-
Fetal Bovine Serum	10%	10%	10%	-
Penicillin & Streptomycin	100U/ml & 100µg/ml	100U/ml & 100 µg/ml	100U/ml & 100 µg/ml	-
Puromycin	1 µg/ml	1 µg/ml	-	-
Recovery Cell culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 Thaw a vial of cells in 9ml of pre-warmed medium and seed them in T75 flask at 2 million cells

1.3. Propagation method

1.3.1 Trypsinize cells from the flask and centrifuge

1.3.2 Add culture medium to the pellet and passage at 3-4 million per T-225 flask

2. Assay Protocol

2.1 Harvest and resuspend cells in culture/assay medium

2.2 Dispense 1500 cells/4µL/well into 1536-well tissue treated black clear bottom plates

2.3 Incubate the plates for 18hr at 37C and 5% CO₂

2.4 Loading 4 µL of buffer with Calcium dye by BioRAPTR

2.5 Incubate the plates for 1hr at 37C and 5% CO₂ and then Incubate at room temperature

2.6 Transfer 23nL of compounds from the library collection and positive control to the assay plates through Pintool of FDSS

2.7 Readout through FDSS 7000EX kinetic plate reader with a filter set of Ex/Em=480/540 for 3 min at 1 sec intervals

2.8 Incubate the plates at room temperature for 2min

2.9 Add 1µL medium containing Kisspeptin10 (8 nM final) through FDSS

2.10 Readout through FDSS 7000EX kinetic plate reader with a filter set of Ex/Em=480/540 for 3 min at 1 sec intervals

3. Assay Performance

Kiss1R	Online Validation Agonist (Mean \pm SD)	Online Validation Antagonist (Mean \pm SD)
EC50	0.31 \pm 0.13 nM (n = 27)	0.24 \pm 0.09 μ M (n = 27)
S/B	9.14 \pm 1.07	22.09 \pm 3.15
CV (%) [*]	31.27 \pm 9.93 (n = 27)	13.45 \pm 2.48 (n = 27)
Z'	0.69 \pm 0.05	0.53 \pm 0.08

^{*}CV values shown represent average of DMSO plates and low concentration plates only.