# Protocol of GnRHR_ HEK293 Cell-based Assay for High-throughput Screening

**DOCUMENT:** GnRH_TOX21_SLP_Version1.0

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## ASSAY RFERENCES:

| Assay Target | Cell Lines | Species | Tissue of Origin | Assay Readout | Assay Provider | Toxicity Pathway |
|---|---|---|---|---|---|---|
| GnRHR (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 |
| G418 (Geneticin) | ThermoFisher Scientific | ThermoFisher Scientific / 10131035 |
| 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 |
| Goserelin (Agonist control compound | MedChemExpress(MCE) | MCE/ HY-13673A |
| Relugolix (Antagonist control compound) | MedChemExpress(MCE) | MCE/HY-16474 |

## 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 | - |
| G418 | 500 µg/ml | 500 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 Goserelin (5 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

| GnRHR (Goserelin; Agonist control) | Online Validation Agonist (Mean ± SD) | Online Validation Antagonist (Mean ± SD) |
|---|---|---|
| EC50 | 1.88 ± 0.65 nM (n = 27) | 5.67 ± 1.64 nM (n = 27) |
| S/B | 28.71 ± 8.67 | 3.53 ± 1.09 |
| CV (%)* | 23.57 ± 7.26 (n = 18) | 12.20 ± 2.31 (n = 18) |
| Z' | 0.65 ± 0.11 | 0.42 ± 0.15 |

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