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

**DOCUMENT:** AR-BLA_TOX21_SLP_Version1.0

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## ASSAY RFERENCES:

| Assay Target | Cell Lines | Species | Tissue of Origin | Assay Readout | Assay Provider | Toxicity Pathway |
|---|---|---|---|---|---|---|
| Androgen receptor : LBD (Recombinant) | HEK293 | Rat (Androgen Receptor) | Embryonic kidney | Beta lactamase reporter | Invitrogen | NR signaling |

## QUALITY CONTROL PRECAUTIONS:

1. -Cell culture is maintained by passaging twice a week and should not reach more than 90% confluence
2. -The assay should be performed in black-clear bottom 1536 well plates, so the bottom of the plates should not be touched

## MATERIALS and INSTRUMENTS:

| Supplies/Medium/Reagent | Manufacturer | Vender/Catalog Number |
|---|---|---|
| -DMEM, high glucose | -Invitrogen | -Invitrogen/11965 |
| -Opti-MEM | -Invitrogen | -Invitrogen/11058 |
| -Dialyzed FBS | -Invitrogen | -Invitrogen/26400 |
| -HEPES | -Invitrogen | -Invitrogen/15630 |
| -NEAA | -Invitrogen | -Invitrogen/11140 |
| -Sodium pyruvate | -Invitrogen | -Invitrogen/11360 |
| -Penicillin and Streptomycin | -Invitrogen | -Invitrogen/15140 |
| -Hygromycin | -Invitrogen | -Invitrogen/10687 |
| -Zeocin | -Invitrogen | -Invitrogen/R250-01 |
| -0.05% Trypsin-EDTA | -Invitrogen | -Invitrogen/25300 |
| -Recovery Cell Culture Freezing Medium | -Invitrogen | -Invitrogen/12648 |
| -Black-clear bottom 1536 well plates | -Greiner | -Greiner/789092F |

| | | |
|---|---|---|
| -LiveBLAzer B/G FRET substrate | -Invitrogen | -Invitrogen/K1028 |
| -Multidrop COMBI | -Thermo Electron Corporation | -Thermo Electron Corporation |
| -BioRAPTR FRD dispenser | -Beckman Coulter | -Beckman Coulter |
| -Envision Plate Reader | -Perkin Elmer | -Perkin Elmer |
| R1881 or Methyltrienolone (Agonist control compound) | -Perkin Elmer | - Perkin Elmer/ NLP005005MG |

## PROCEDURE:

1. Cell handling:
   1.1. Media Required:

| Component | Growth Medium | Assay Medium | Thaw Medium | Freezing Medium |
|---|---|---|---|---|
| -DMEM, high glucose | -90% | - | -90% | - |
| -Opti-MEM | - | -90% | - | - |
| -Dialyzed FBS | -10% | -10% | -10% | - |
| -HEPES | -25mM | - | -25mM | - |
| -NEAA | -0.1mM | -0.1mM | -0.1mM | - |
| -Sodium pyruvate | -1mM | -1mM | -1mM | - |
| -Penicillin and Streptomycin | -100U/ml and 100ug/ml | -100U/ml and 100ug/ml | -100U/ml and 100ug/ml | - |
| -Hygromycin | -80ug/ml | - | - | - |
| -Zeocin | -80ug/ml | - | - | - |
| -Recovery Cell Culture Freezing Medium | - | - | - | -100% |

   1.2. Thawing method

      1.2.1 -1ml frozen cells of AR-bla were taken in pre-warmed 10ml of thaw medium for centrifuging
      1.2.2 -Thaw medium is used to re-suspend the pellet
      1.2.3 -Seed the cells at 2 million per T-75 flask with thaw medium

   1.3. Propagation method

      1.3.1 -Detach the cells from the flask using 0.05% Trypsin
      1.3.2 -The cells are re-seeded in T-225 flask at 3-4 million

2. Assay Protocol

   2.1 -Spin down the cells after rinsing the cells with DPBS and trypsinizing

2.2 -Resuspend the pellet with assay medium
2.3 -Plate the cells in black-clear bottom 1536 well plate at 2000/well/6uL through 8 tip Multidrop plate dispenser
2.4 -Incubate at 37C for 5hrs
2.5 -Transfer 23nL of compounds from the library collection and positive control to the assay plates through Pintool
2.6 -Incubate at 37C for 16hrs
2.7 -Add 1uL of CCF4 (FRET Substrate) dye using a single tip plate dispenser (Bioraptr)
2.8 -Incubate at room temperature for 2hrs
2.9 -Read the fluorescence intensity through Envision plate reader

3. Assay Performance

| **AR-bla (R1881; Agonist control)** | **Online Validation Agonist (Mean ± SD)** |
|---|---|
| EC50 | 1.06 ± 0.10 nM (n = 27) |
| S/B | 2.07 ± 0.18 |
| CV (%)* | 6.67 ± 2.36 (n = 18) |
| Z' | 0.34 ± 0.13 |

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