# Protocol of P53-BLA HCT-116 Cell-based Assay for High-throughput Screening

**DOCUMENT:** P53-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 |
|---|---|---|---|---|---|---|
| P53 (Recombinant) | HCT-116 | Human | Colon carcinoma | Beta-lactamase reporter | Invitrogen | Stress response |

## QUALITY CONTROL PRECAUTIONS:

1. Handle the 1536-well, black-wall, clear-bottom assay plate by the sides; do not touch the clear bottom of the assay plate.

## MATERIALS and INSTRUMENTS:

| Supplies/Medium/Reagent | Manufacturer | Vender/Catalog Number |
|---|---|---|
| McCoy's 5A Medium | Invitrogen | 16600 |
| Opti-MEN Reduced Serum Medium | Invitrogen | 11058 |
| Fetal bovine serum, dialyzed | Invitrogen | 26400 |
| Nonessential amino acids (NEAA) | Invitrogen | 11140 |
| Penicillin/Streptomycin (antibiotic) | Invitrogen | 15140 |
| DPBS | Invitrogen | 14190 |
| Sodium pyruvate | Invitrogen | 11360 |
| 0.25% Trypsin/EDTA | Invitrogen | 25300 |
| Blasticidin (antibiotic) | Invitrogen | R210 |
| DMSO | AMRESCO | KD Medical, RGE-3070 |
| Recovery Cell Culture | Invitrogen | 12648 |
| LiveBLAzer FRET B/G Loading Kit | Invitrogen | K1030 |
| Solution D | Invitrogen | K1157 |
| Mitomycin C | Calbiochem | 475820 |
| Nutlin-3 | Calbiochem | 444143 |

| | | |
|---|---|---|
| Black-wall, clear-bottom, 1536-well assay plates | Greiner Bio-One | 789092-F |
| PinTool | Kalypsys | - |
| BioRAPTR, Microfluidic Workstation | Beckmen | - |
| EnVision plate reader | Perkin Elmer | - |
| Centrifuge | Sorvall legend XTR | Thermo Fisher Science, 75004520 |

## PROCEDURE:

1. Cell handling:
   1.1. Media Required:

| Component | Growth Medium | Assay Medium | Thaw Medium | Freezing Medium |
|---|---|---|---|---|
| McCoy's 5A medium | 90% | - | 90% | - |
| Opti-MEN | - | 99.5% | - | - |
| Dialyzed FBS | 10% | 0.5% | 10% | - |
| NEAA | - | 0.1 mM | - | - |
| Sodium pyruvate | - | 1mM | - | - |
| Penicillin/Streptomycin | 100U/mL/100µg/mL | 100U/mL/100µg/mL | 100U/mL/100µg/mL | - |
| Blasticidin (antibiotic) | 5 µg/mL | - | - | - |
| Recovery Cell Culture | - | - | - | 100% |

1.2. Thawing method

1.2.1 Place 14 mL of pre-warmed thaw medium into a 15 mL of conical tub.
1.2.2 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.
1.2.3 Decontaminate the vial by wiping with 70% ethanol before opening in a biological safety cabinet.
1.2.4 Transfer the vial contents drop-wise into 14 mL of thaw medium in a sterile 15-mL conical tub.
1.2.5 Centrifuge cells at 900 rpm for 4 minutes and resuspend in thaw medium.
1.2.6 Transfer contents to the T75 tissue culture flask containing Thaw Medium and place flask in a humidified 37°C/5% CO2 incubator.
1.2.7 Switch to growth medium at first passage.

1.3. Propagation method

1.3.1 Aspirate medium, rinse once in DPBS, add 0.25% Trypsin/EDTA and swirl to coat the cell evenly.
1.3.2 Add an equal volume of Growth Medium to inactivate Trypsin after 2-3 minutes incubation at 37°C.
1.3.3 Centrifuge cells at 900 rpm for 4 minutes and resuspend in growth medium.
1.3.4 Cell should be passage or fed at least twice a week.

2. Assay Protocol

2.1 Harvest cells from culture in growth medium and resuspend in assay medium.
2.2 Dispense 4000 cells/5 µL/well into 1536-well black/clear bottom plates using a Multidrop dispenser.
2.3 After the cells were incubated at 37°C for 5 hours, 23 nL of compounds dissolved in DMSO were transferred to the assay plate by a PinTool resulting in a 217-fold dilution
2.4 Incubate the plates for 16 hours at 37°C, 5% CO2.
2.5 Add 1 µL of 6X LiveBLAzer FRET B/G (CCF4-AM) Substrate Mixture to each well using a BioRAPTR dispenser and incubate the plates at room temperature for 2 hours.
2.6 Measure fluorescence intensity at 460 and 530 nm emission and 405 nm excitation by an Envision detector. Data is expressed as the ratio of 460nm/530nm emissions.
2.7 Add 4 uL of Cell-titer Glo to each well using BioRAPTR and incubate the plates at room temperature for 2 hours
2.8 Measure fluorescence intensity by a VewLux plate reader.

3. Assay Performance

| p53RE-bla Agonist (Mitomycin C) | Online Validation (Mean ± SD) |
|---|---|
| EC50 | 3.50 ± 0.73 µM (n = 27) |
| S/B | 3.08 ± 0.26 (n = 27) |
| CV (%)* | 5.58 ± 0.70* (n=18) |
| Z' | 0.70 ± 0.05 (n = 27) |

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