Protocol of DNA damage-chicken-DT40 B lymphocyte Cell-based Assay for High-throughput Screening

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| **DOCUMENT:** |  | DNA damage-chicken-DT40\_TOX21\_SLP\_Version1.0 |
| **TITLE:** |  | Protocol of DNA damage-chicken-DT40 B lymphocyte Cell-based Assay for High-throughput Screening |

**ASSAY RFERENCES:**

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| --- | --- | --- | --- | --- | --- | --- |
| Assay Target | Cell Lines | Species | Tissue of Origin | Assay Readout | Assay Provider | Toxicity Pathway |
| DNA repair  (Endogenous/knockdown) | B lymphocyte | Chicken | B lymphocyte | Luminescence | Dr. Takeda | Stress response |

**QUALITY CONTROL PRECAUTIONS:**

1.

**MATERIALS and INSTRUMENTS:**

|  |  |  |
| --- | --- | --- |
| Supplies/Medium/Reagent | Manufacturer | Vender/Catalog Number |
| RPMI1640 | Invitrogen | 11875 |
| Heated Inactive FBS | Sigma | F4135 |
| Penicillin/Streptomycin | Invitrogen | 15140 |
| 2-Mercaptoethanol | Invitrogen | 21985 |
| Recovery Cell Culture | Invitrogen | 12648 |
| CellTiter Glo | Promega | G7573 |
| White 1536-well assay plates | Greiner BioOne | 789073-F |
| Chicken serum | Invitrogen | 16110 |
| Tetraoctylammonium bromide (Tetra Br.) | Sigma | 294136 |

**PROCEDURE:**

1. Cell handling:

1.1. Media Required:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Growth Medium | Assay Medium | Thaw Medium | Freezing Medium |
| RPMI1640 | 89% | 89% | 89% | - |
| Heated Inactive FBS | 10% | 10% | 10% | - |
| Chicken serum | 1% | 1% | 1% | - |
| 2-Mercapto ethanol | 50uM | 50uM | 50uM | - |
| Penicillin/Streptomycin | 100U/mL/100µg/mL | 100U/mL/100µg/mL | 100U/mL/100µg/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 tube.

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 12 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 dropwise into 14 mL of thaw medium in a sterile 15mL 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 Growth Medium and place flask in a humidified 37°C/5% CO2 incubator.

1.3 Propagation method

1.3.1 Cells were cultured as a suspension in a humidified atmosphere with 5% CO2 at 37°C.

1.3.2 Cultures can be maintained by addition or replacement of fresh medium every day.

2. Assay Protocol

2.1 Harvest cells and resuspend in growth medium.

2.2 Dispense cells at 300 cells/5µL/well into 1536-well, white plates using a mutidrop dispenser.

2.3 After the cells were plated, 23 nL of control or compounds dissolved in DMSO, positive controls or DMSO were transferred to the assay plate by a PinTool resulting in a 217-fold dilution.

2.4 The final compound concentration in the 5 µl assay volume ranged from 1.2 nM to 92 µM in 15 concentrations.

2.5 Incubate the plates for 40 hours at 37°C.

2.6 Add 5 µL of CellTiter-Glo to each well using a BioRAPTR dispenser and incubate the plate at room temperature for 30 minutes.

2.7 Measure luminescent intensity by a ViewLux.

3. Assay Performance

|  |  |
| --- | --- |
| DT40-100  (Tetra Br.) | Online Validation  (Mean ± SD) |
| IC50 | 0.39 ± 0.08 μM  (n =27) |
| S/B | 47.00 ± 0.70  (n = 27) |
| CV (%) | 9.75 ± 2.42\*  (n=18) |
| Z’ | 0.66 ± 0.04  (n = 27) |

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

|  |  |
| --- | --- |
| DT40-657  (Tetra Br.) | Online Validation  (Mean ± SD) |
| IC50 | 0.37 ± 0.08 μM  (n = 27) |
| S/B | 45.00 ± 2.60  (n = 27) |
| CV (%) | 15.19 ± 4.02\*  (n=18) |
| Z’ | 0.87 ± 0.017  (n = 27) |

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

|  |  |
| --- | --- |
| DT40-653  (Tetra Br.) | Online Validation  (Mean ± SD) |
| IC50 | 0.21 ± 0.014 μM  (n = 27) |
| S/B | 55.0 ± 2.20  (n = 27) |
| CV (%) | 10.61 ± 1.97\*  (n=18) |
| Z’ | 0.88 ± 0.02  (n = 27) |

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