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

DOCUMENT: DNA damage-chicken-DT40_TOX21_SLP_Version1.0

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ASSAY RFERENCES:

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