Protocol of HDAC I/II HCT-116 Cell-based Assay for High-throughput Screening

DOCUMENT: HDAC I/II_TOX21_SLP_Version1.0

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ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Histone Deacetylase class I and II	HCT-116	Human	Colorectal carcinoma	Luminescence	Promega	Histone Deacetylases

QUALITY CONTROL PRECAUTIONS:

1. -Cell culture is maintained by passing the cells twice a week and should not reach more than 90% confluence

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-McCoys 5a Medium	-ATCC	-ATCC/30-2007
-Fetal Bovine Serum	-ATCC	-ATCC/30-2020
-Penn-strep	-Invitrogen	-Invitrogen/15140
-Trichostatin A (Positive control compound)	-Selleckchem	-Selleckchem/S1045
-Tetraoctyl ammonium bromide (Positive control for Cytotoxicity)	-Sigma Aldrich	-Sigma Aldrich/294136
-Recovery Cell Culture Freezing Medium	-Invitrogen	-Invitrogen/12648
-0.25% Trypsin-EDTA	-Invitrogen	-Invitrogen/25200
-Triton X-100	-Sigma Aldrich	-Sigma Aldrich/T8787
-HDAC-Glo(TM) I/II Assay	-Promega	-Promega/G6422
-CellTiter-Glo(R) One Solution Assay	-Promega	-Promega/G8462
-BioRAPTR FRD dispenser	-Beckman Coulter	-Beckman Coulter
-White-solid bottom, Tissue treated 1536-well assay plates	-Greiner Bio-one	-Greiner Bio-one/789173-F
-Multidrop COMBI	-Thermo Electron Corporation	-Thermo Electron Corporation
-ViewLux Plate Reader	-Perkin Elmer	-Perkin Elmer

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-McCoys 5a Medium	-90%	-90%	-90%	-
-Fetal Bovine Serum	-10%	-10%	-10%	-
-Penn-strep	-100U/ml-100ug/ml	-100U/ml-100ug/ml	-100U/ml-100ug/ml	-
-Recovery Cell Culture Freezing Medium	-	-	-	-100%

1.2. Thawing method

1.2.1 -Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.
1.2.2 -Take 1mL of frozen HCT-116 cells in pre-warmed 9ml of thaw medium and centrifuge.
1.2.3 -The cells were seeded in T-75 flask at 2 million cells

1.3. Propagation method

1.3.1 -Aspirate medium, rinse twice in DPBS and add 0.25% Trypsin/EDTA (3 mL for a T75 flask) 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 37C and then collect it into a tube for centrifugation.
1.3.3 -The cells can be further passed to the next passage if required.

2. Assay Protocol

2.1 -Harvest cells from the culture and make it to a density of $0.3X10^6$ cells/mL.
2.2 -Dispense 1500 cells in 5uL of the culture medium per well into 1536-well tissue treated white solid bottom plates using a Multidrop Combi dispenser.
2.3 -Incubate at 37C for an overnight (18-20hrs).
2.4 -Transfer 23nL of compounds from the library collection into 5-48 columns and positive control into 1-4 columns using a Pintool station.
2.5 -Incubate at 37C for 2hrs.
2.6 -Add 4uL of CellTiter-Glo reagent using a single tip of a BioRAPTR dispenser.
2.7 -Incubate at room temperature for 30min
2.8 -Read the luminescence intensity using ViewLux plate reader (Exposure time = 1sec).