Top2 inhibitor sensitivity

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1 Works for me dx.doi.org/10.17504/protocols.io.k5zcy76

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ABSTRACT

Protocol for Top2i sensitivity in attached cells in 96-well format. Short term exposure to Top2i (~3 days)

MATERIALS

NAME	CATALOG #	VENDOR
50 mg Doxorubicin HCl	orb340472	biorbyt
500 mg Etoposide	orb322762	biorbyt
Doxycycline Hyclate	D-500	Gold Biotechnology

SAFETY WARNINGS

TRPz lenti-vrius is BSL2

BEFORE STARTING

Using a Tripz doxyrubicin inducible shRNA system that is already in transduced cells.

1 Plate (2) 6cm dishes at 25% confluency (approx. 500K cells). Let attach overnight

Induction of shRNAs

2 Add 1000x Doxycyclin to 1 plate of cells with fresh media and induce for 72 hours changing media with fresh doxycyclin daily. Also change media to corresponding non-induced plate

72:00:00

In 96-well plates

14h

3 Split cells at density appropriate for cell type. (eg. 5000k cells/well) in total of 150ul of media per well.
Make master mixes of cells/well in 50ml conicals and distribute using multi-channel pipette.
Add doxyxyclin to induced samples at 500x
Let cells attach overnight

14:00:00

4
- Dilute Top2i in media 96-well round bottom plate for transfer to plates with cells
- Dilution in media without Doxycycline so that both cell samples can get Top2i from the same dilutions
- In our case 2ul of stock concentration of drug (see making stock plate) in 250ul of media (1:125) --> (1:500 when 50uls is added to 150ul already in well.
- Doxycycline concentration will be diluted in induced samples but was starting with a higher concentration).
- One row is enough for 1/2 plate. Make multiple rows of dilutions for more plates.

5 **48:00:00**

Incubate cells with Top2i for 2 days

6
- Dilute cell titer blue 1:2 in PBS
- Add 20ul of cell titer blue to wells and let sit overnight

14:00:00

7
- Transfer suppernatant to 384-well black plates for reading
- Excite at 544nm and emission of 590nm