

QL13 QL13-67

Done

Title: QL13-67

Goal: PTO Cy5 uptake, 500 nM, inhibition MMTS, IPS, 2 plates

Started on: May 4, 2021

Modified on: June 9, 2021

Closed date

Project: Phosphorothioates

Type: PTO Cy5 Inhibition

HeLa Kyoto cells were seeded at 8×10^4 cells/mL in FluoroBrite DMEM + 10% FBS on μ -Plates 96-well ibiTreat sterile and kept at 37 °C with 5% CO₂ overnight. The following day, cells were washed with FluoroBrite DMEM (3 × 3 mL/well) using a plate washer, keeping a final volume of 135 μ L/well.

Dilutions of Cy5-labelled phosphorothioate and inhibitor in PBS were prepared in a 96-well V-bottom plate and added to the μ -Plate containing the cells at the desired time (15 μ L/well, 10x final concentration in PBS) to reach a final volume of 150 μ L/well, and cells were incubated for 1 h at 37 °C with 5% CO₂ in DMEM. Cells were washed with FluoroBrite DMEM (3 × 3 mL/well) using a plate washer, keeping a final volume of 135 μ L/well, and the corresponding solution of phosphorothioate was added (15 μ L/well, 10x final concentration in PBS) to reach a final volume of 150 μ L/well, and cells were incubated for 2 h at 37 °C with 5% CO₂ in DMEM.

Then, Hoechst 33342 (15 μ L/well, 100 μ g/mL in PBS) was added. After 15 min of incubation at 37 °C with 5% CO₂, the medium was removed and cells were fixed with a PFA solution (3% in PBS, 80 μ L/well) for 20 min. The plate was washed with PBS (3 × 3 mL/well) and imaged using a high-content automated microscope.

PLATE 1

Sequence: AGGTCCCACATACACCGAC

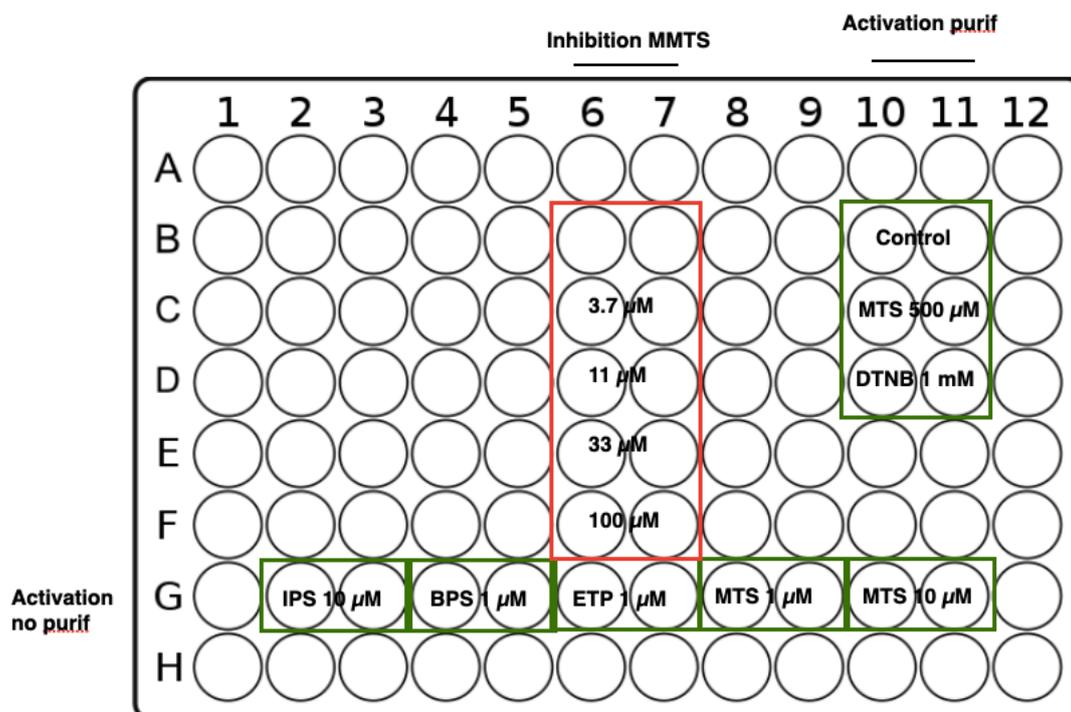
1 h pre-incubation, 2 h incubation, 37 °C

OPS 500 nM



	1	2	3	4	5	6	7	8	9	10	11	12
A												
No inhibitor												
3.7 μ M												
11 μ M												
33 μ M												
100 μ M												
300 μ M												
H												

PLATE 2



Attached files

QL13-01-Inhibition.pzfx
 Capture d'écran 2021-06-09 à 14.21.29.png
 QL13-01-Activation.pzfx
 QL13-01-Plates.key