

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  $8 \times 10^4$  cells/mL in FluoroBrite DMEM + 10% FBS on  $\mu$ -Plates 96-well ibiTreat sterile and kept at 37 °C with 5% CO<sub>2</sub> overnight. The following day, cells were washed with FluoroBrite DMEM ( $3 \times 3$  mL/well) using a plate washer, keeping a final volume of 135  $\mu$ L/well.

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

Then, Hoechst 33342 (15  $\mu$ L/well, 100  $\mu$ g/mL in PBS) was added. After 15 min of incubation at 37 °C with 5% CO<sub>2</sub>, the medium was removed and cells were fixed with a PFA solution (3% in PBS, 80  $\mu$ L/well) for 20 min. The plate was washed with PBS ( $3 \times 3$  mL/well) and imaged using a high-content automated microscope.

PLATE 1

Sequence: AGGTCCCACATACACCGAC

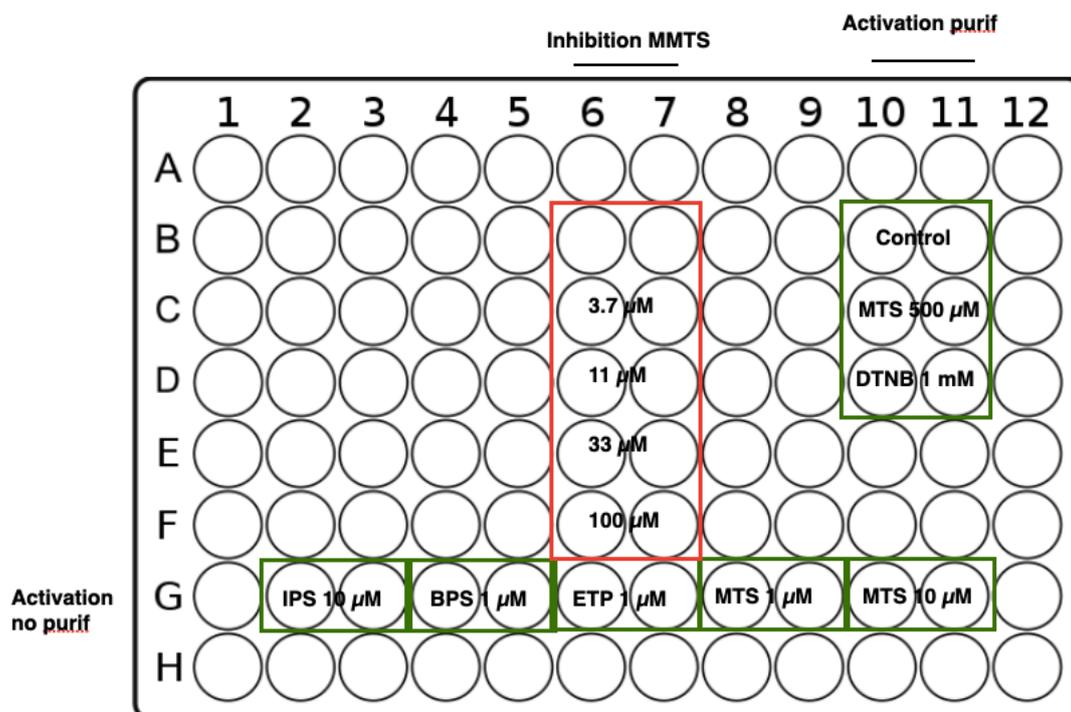
1 h pre-incubation, 2 h incubation, 37 °C

OPS 500 nM



|              | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------------|---|---|---|---|---|---|---|---|---|----|----|----|
| A            |   |   |   |   |   |   |   |   |   |    |    |    |
| No inhibitor |   |   |   |   |   |   |   |   |   |    |    |    |
| 3.7 $\mu$ M  |   |   |   |   |   |   |   |   |   |    |    |    |
| 11 $\mu$ M   |   |   |   |   |   |   |   |   |   |    |    |    |
| 33 $\mu$ M   |   |   |   |   |   |   |   |   |   |    |    |    |
| 100 $\mu$ M  |   |   |   |   |   |   |   |   |   |    |    |    |
| 300 $\mu$ 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