

QL12 QL12-20

Done

Title: QL12-20

Goal: PTO Cy5 uptake, 500  $\mu$ M, 2 h coincubation with 8 different inhibitors

Started on: January 22, 2021

Modified on: June 9, 2021

Closed date

Project: Phosphorothioates

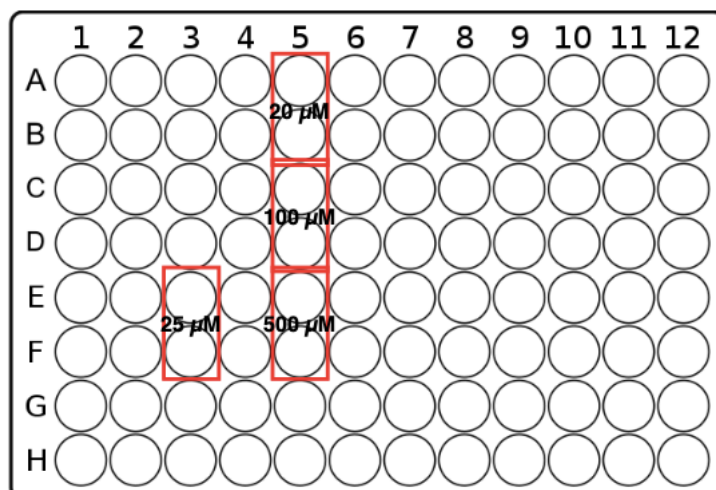
Type: PTO Cy5 Inhibition

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 6.5 h at 37 °C with 5% CO<sub>2</sub> in DMEM.

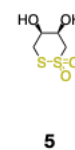
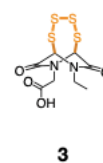
Then, Hoechst 33342 (15  $\mu$ L/well, 100  $\mu$ g/mL in PBS) was added. After 15 min of incubation at 37 °C with 5% CO<sub>2</sub>, the medium was removed and cells were fixed with a PFA solution (3% in PBS, 80  $\mu$ L/well) for 20 min. The plate was washed with PBS (3  $\times$  3 mL/well) and imaged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

Sequence: AGGTCCCCATACACCGAC

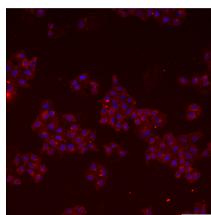


Activation 30 min

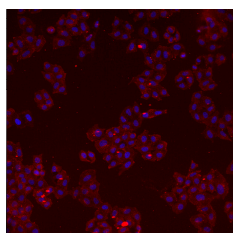
2 h incubation, 37 °C



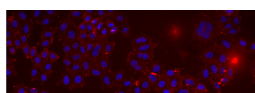
A5

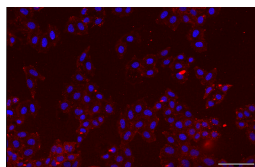


C5

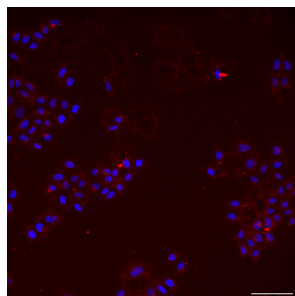


E5





E3



## Attached files

[QL12-20 Plate.pdf](#)[C5.png](#)[B5.png](#)[D5.png](#)[G5.png](#)[F3.png](#)[E3.png](#)[E5.png](#)[F5.png](#)