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**Username :** Quentin Laurent

## QL12 QL12-63

Done

**Title:** QL12-63

**Goal:** PTO Cy5 uptake, 500 nM, 1 h preincubation + 2 h incubation with different inhibitors, activation

**Started on: February 25, 2021**

**Modified on: June 9, 2021**

*Closed date*

**Project:** Phosphorothioates

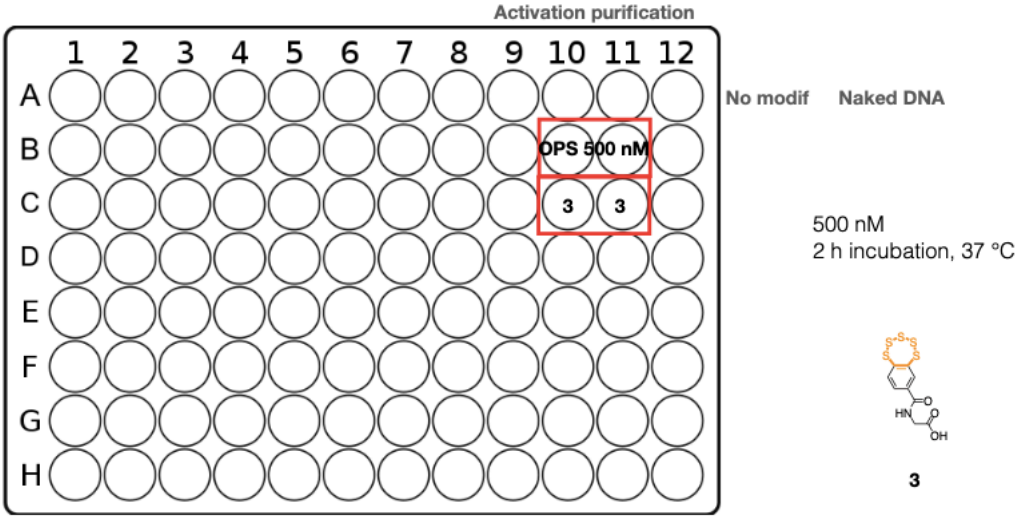
**Type:** PTO Cy5 Inhibition

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% $CO_2$ overnight. The following day, cells were washed with FluoroBrite DMEM (3 × 3 mL/well) using a plate washer, keeping a final volume of 135 µL/well.

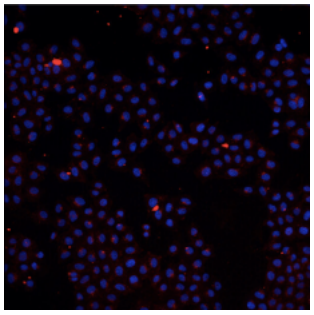
Dilutions of Cy5-labelled phosphorothioate and inhibitor in PBS were prepared in a 96-well V-bottom plate and added to the µ-Plate containing the cells at the desired time (15 µL/well, 10x final concentration in PBS) to reach a final volume of 150 µL/well, and cells were incubated for 1 h at 37 °C with 5% $CO_2$ in DMEM. Cells were washed with FluoroBrite DMEM (3 × 3 mL/well) using a plate washer, keeping a final volume of 135 µL/well, and the corresponding solution of phosphorothioate was added (15 µL/well, 10x final concentration in PBS) to reach a final volume of 150 µL/well, and cells were incubated for 2 h at 37 °C with 5% $CO_2$ in DMEM.

Then, Hoechst 33342 (15 µL/well, 100 µg/mL in PBS) was added. After 15 min of incubation at 37 °C with 5% $CO_2$, the medium was removed and cells were fixed with a PFA solution (3% in PBS, 80 µL/well) for 20 min. The plate was washed with PBS (3 x 3 mL/well) and imaged using a high-content automated microscope.

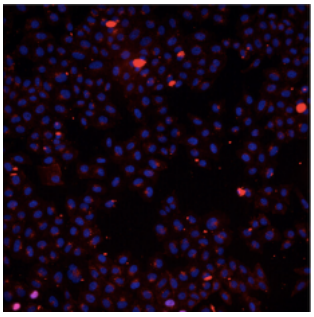
Sequence: AGGTCCCCATACACCGAC




B11



C10



Attached files

- Capture d'écran 2021-06-09 à 14.29.07.png
- C10.png
- B11.png
- QL12-63 Plate.pdf