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Username : Quentin Laurent

QL13 QL13-11

Done

Title: QL13-11

Goal: PTO Cy5 uptake, 500 nM, activation + activation followed by dialysis with 5 compounds

Started on: March 18, 2021

Modified on: May 12, 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.

Attached files

- QL13-01-Plates.key
- QL13-01-Activation.pzfx
- QL13-01-Inhibition.pzfx