H2020-MSCA-ITN-2020

Allostery in Drug Discovery

GloSensor cAMP assay

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Abstract: The GloSensor cAMP assay is an activity assay for GPCR coupled to Gs proteins, in which the cAMP levels are measured in live cells via bioluminescence.

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The GloSensor cAMP assay measures the activity of G-protein coupled receptors (GPCRs) through their effect on the Gs protein, and specifically the downstream increase of cAMP. It does so using a luciferase biosensor, whose binding to cAMP results a conformational shift that promotes large increases in luminescence in a manner directly proportional to the amount of cAMP present.

This assay has proven to work very successfully with the MC3R and MC4R proteins along with several MC4R mutants. However, it is worth noting that γ2-MSH (an endogenous ligand of MC3R) showed unspecific background GloSensor signal at high concentrations. Interestingly, this was not the case for the very similar ligand γ1-MSH despite their only difference being the presence of amidation, a post-translational modification, on the C-terminal end of γ1-MSH, whereas on γ2-MSH the C-terminal ends on a glycine residue. Thus, it is recommended to be cautious with the testing of this ligand as well as other ligands with exposed terminal glycines.

Protocol:

Around 24h before the start of the experiment the HEK293T cells should be transfected with the p22-GloSensor plasmid from Promega and the target protein, then placed in the whole 96-well plate except for column 1, which will contain only the plasmid without the protein as a negative control. Keep the plate in the incubator at 37°C.

- A luciferase solution is prepared with 400µl of luciferase diluted in 10ml OptiMEM (per plate)
- The plate is taken out of the incubator and the cell medium in each of its wells is carefully and completely aspirated.
- 90 µl of the luciferase solution is placed in each well using a multichannel pipette.
- This measurement plate is then covered by aluminum foil then placed back in the incubator at 37°C for 2h.
- In the meantime, make the dilution plate:
- Place 50 µl OptiMEM in each well of columns 1 and 3 (negative controls), place 50 µl of forskolin (typically 100 µM) in column 2 (positive control), place 55 µl of the ligands to be tested (3 ligands can be tested in triplicate per plate) in row H starting at column 4 at appropriate start concentrations (typically 1 mM) and place 45 µl OptiMEM in the remaining wells.
- Then perform the serial dilution step by taking 5 µl of row H from column 4 (the ligands) to G with a multichannel pipette, pipetting up and down then switching tips and repeating the process from G to F, F to E etc… until B to A.

- Cover this plate in a plastic sheet then briefly spin this plate in a concentrator to ensure that the contents are at the bottom of the wells and leave it in the fridge. Note that one dilution plate can be used to do about 4 measurement plates.
- When the 2h have lapsed, take the plates out of the incubator and the fridge respectively and transfer 10 µl from each well from the dilution plate to the measurement plate using a multichannel pipette starting from row A and moving down as quickly as possible.
- Start a timer and take measurements at a wavelength of 545-50 nm after at least 15 min have lapsed. Make sure to keep any additional plates as warm as possible in the meantime as colder temperatures have been observed to result in a higher level of background signaling.

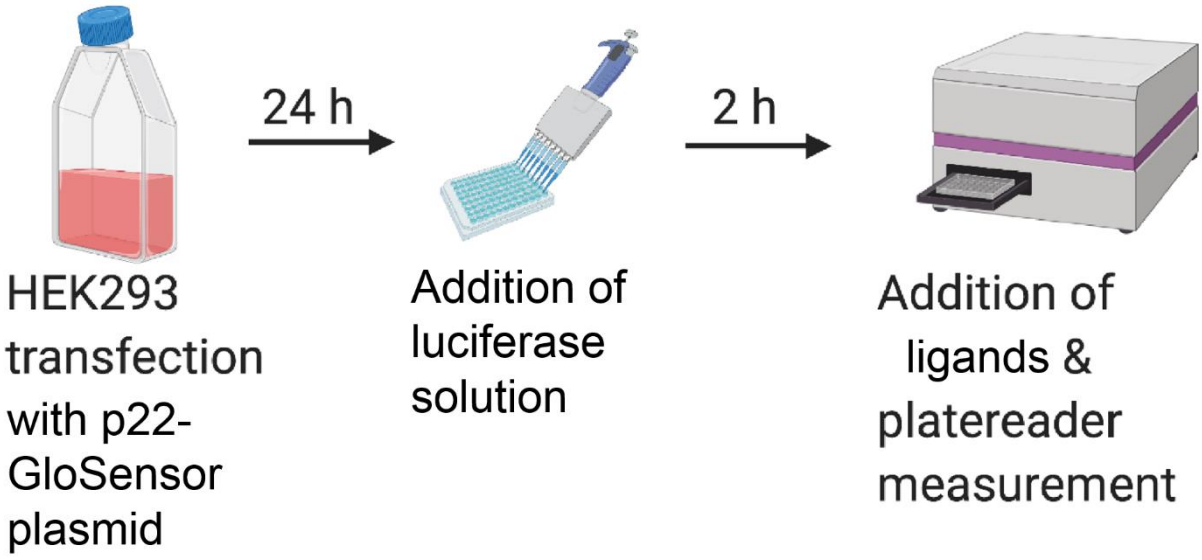

Figure 2: GloSensor cAMP assay workflow.