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Actiflash

Q&A

Q/ Actiflash was tested on what type of biological samples?

A: Actiflash has been successfully used with cell lines as well as animal models:

- Cell lines: CV1 cell line, mammalian epithelial cells (MDCK)
- 12 hpf – 3 dpf zebrafish embryos
- Mice.

Q/ Do you have examples of Protein-ERT constructs that were previously used with caged Cyclofen-OH?

A: You will find below a non-exhaustive list of proteins that have been fused to the estrogen receptor:

- Recombinase Cre
- Tyrosine-protein kinase ABL1
- Cellular tumor antigen p53
- Proto-oncogene c-Fos
- Endothelial transcription factor GATA-2
- CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase4 (GAL4).

Q/ How do I store Actiflash?

A: Always wrap the vials containing the aliquoted Actiflash with aluminium foil.

Actiflash is provided as a powder. It is stable for years in the dark at 2-8°C.

Once solubilized in a DMSO solution (typically at 10 mM), it must be stored in the dark at -20°C, where it is stable for several months.

Q/ How do I check my protein-ERT construction?

A: You can perform experiments with Tamoxifen-OH, as a positive control.

Q/ How do I check the photoactivation efficiency?

A: After conditioning your sample at a given concentration (e.g. 5 µM) with Actiflash, illuminate your samples with the shortest illumination duration leading to the maximum phenotype recovery. Compare with the results obtained with Tamoxifen-OH (1 µM).

Q/ Which plasmid ERT do you recommend?

A: Any plasmid containing the ERT sequence should work.

Q/ How can I determine the required duration of illumination that will activate Actiflash with my samples?

A: With the Actiflash conditioned samples, analyze the phenotype recovery observed using a 100% laser power and a decreasing illumination duration. You will thus determine the shortest illumination duration leading to 100% uncaging.

Examples of the duration required for uncaging 100% of Actiflash:

- One-photon excitation at a 365 nm wavelength with a benchtop UV lamp having a power range of 4-6 W (e.g. for global photoactivation): 5 minutes.
- One-photon excitation in the 350-405 nm, laser excitation power $P = 10 \mu\text{W}$ focused on a spot of diameter 10 μm : a few seconds.
- Two-photon excitation delivering 200 fs pulses at 750 nm and $P = 10 \text{ mW}$ in a cell of volume $V = 100\text{--}1000 \mu\text{m}^3$: 1 second.

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