Actiflash

PROTOCOL

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com info@eaglebio.com

EAGLE

idylle

PROTOCOL

1. The material you need

- ✔ Actiflash
- ✔ (Z)-4-Hydroxytamoxifen (CAS Number 68047-06-3)
- ✔ Petri dishes
- ✔ Pipetman and tips
- ✔ Spectrophotometric grade DMSO
- ✔ Protein-ERT construct (e.g. Cre-ERT)
- ✔ Biological samples expressing the target of the protein-ERT construct (ex: Lox)
- ✔ Culture medium without serum if your samples are cell lines
- ✔ Aluminium foil
- ✔ Deionized water, ethanol 95%, acetone and dichloromethane if you use glassware
- ✔ Benchtop UV lamp (e.g. 365 nm, 6 W, Fischer-Bioblock) or light source installed on your microscope

- **In the dark:** refers to a total absence of light.
- **Away from light:** refers to samples to be handled outside of direct light (half-light).

2. Glassware washing

- 💡 **It is recommended to use single-use dishes. If it is not possible, it is recommended to use the following protocol to wash the glassware between two experiments using Actiflash.**
- Rinse glassware with deionized water, then successively with ethanol 95%, acetone and finally dichloromethane.

3. Storage

- 💡 **A poor conservation may yield degradation: always store Actiflash in the dark.**
- Actiflash is stable as a powder for years at 2-8°C.
- The Actiflash DMSO solution (typically at 10 mM) must be stored at -20°C and it is stable for several months at this temperature.

- 💡 **Only unfreeze the aliquots that will be used for your daily experiment.**

4. Solubilisation

- Actiflash is provided as a powder in a glass vial.

- In order to obtain a stock solution, the powder must be solubilized at 10 mM in spectrophotometric grade DMSO in the glass tube. For vials containing 5mg of Actiflash, use the volume of DMSO indicated on the label of the vial.
- 💡 **Always check that the powder is well dissolved.**
- ❗ **Be careful because the powder is electrostatic. So, please do not wear gloves that increase electrostaticity.**

- This solution must then be aliquoted in transparent standard Eppendorfs (1 Eppendorf for 1 or 2 experiments) shielded from light with an aluminum foil and stored at -20°C **in the dark.**

- 💡 **Your stock solution of Actiflash in DMSO may solidified at -20°C. Before using it, homogenize the thawed stock DMSO solution with a pipette to ensure that it is homogeneous.**

5. Sample conditioning: Calibration of the Actiflash concentration

Before starting your experiments with Actiflash in order to estimate the adequate concentration of caged Cyclofen-OH, as well as checking that the ERT-protein construct works: It is highly recommended to conduct a first experiment with varying concentrations of (Z)-4-hydroxytamoxifen which

measures the extent of phenotype sought for as a function of the (Z)-4-Hydroxytamoxifen concentration. Then the concentration of Actiflash to be used for sample conditioning is fixed at five times the smallest (Z)-4-Hydroxytamoxifen concentration causing 100% of the desired phenotype (in general 3-6 µM in cultured cells and zebrafish embryos).

- Incubate your samples with increasing concentrations of (Z)-4-hydroxytamoxifen from 0.1 up to 6 µM in the culture medium.

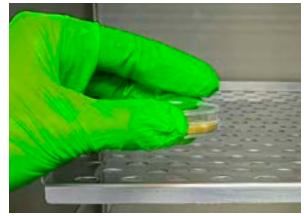
- Incubate for 90 minutes to ensure diffusion of (Z)-4-hydroxytamoxifen in your samples.
- Proceed to phenotype evaluation and select five times the smallest concentration causing 100% of the desired phenotype to fix Actiflah concentration for sample conditioning.

6. Sample conditioning: Conditioning protocol

Serum proteins can sequester Actiflash and hamper its diffusion into biological samples. Hence a serum-free medium must be used during the incubation step of cell lines. After illumination and Actiflash photoactivation, it is possible to switch back to a serum proteins-containing medium.This protocol has been used with cell lines and Zebrafish embryos aged up to 2 days post-fertilization.

- Incubate your samples (cell lines, zebrafish embryos…) in a serum-free medium, at a final concentration of 3-6 µM of Actiflash as determined in the "Calibration of the Actiflash concentration" step.
- **Away from light**, stir manually or mix with a pipetman's tip after adding the stock solution, to promote a good dispersion of the Actiflash solution into the medium.

- Incubate for 90 minutes to ensure the best diffusion of Actiflash throughout your samples, **in the dark** to avoid any photoactivation of Actiflash.



- **In the dark**, just before photoactivation, wash your samples (cell lines, zebrafish embryos …) to make the external medium free of Actiflash (not necessary for two photon laser experiments).
- Illuminate according to the protocol described in the "Actiflash photoactivation" protocol.
- If required, switch back to a serum proteins-containing medium once Actiflash has been photoactivated.

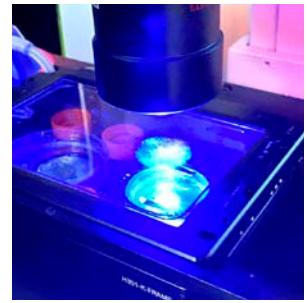
7. Actiflash photoactivation

Illumination of Actiflash may be performed with UV (325-425 nm range) light or multiphoton excitation (at 750 and 1064 nm with two- and three-photon excitation, respectively) to release Cyclofen-OH. Illumination and subsequent activation of Actiflash may be achieved using:

Benchtop UV lamps generally used to read-out thin layer chromatography plates in the chemical laboratory to photoactivate biological samples over large areas. (e.g. Ref: 365 nm, 6 W, Fischer-Bioblock).

Light sources installed on microscopes:

- Between 325 and 425 nm with one-photon excitation. Ref: Microscope with a x25 (or x40) water immersion objective (Leica or Olympus) equipped for local activation with a Hg lamp or a UV laser line (e.g. from a 365 nm CW; Crystal Laser).
- Between 700 and 800 nm with two-photon excitation (200 fs, 76 MHz, 750 nm, provided by a mode-locked Ti-Sapphire laser, Mira-Coherent) illuminating the samples via a x25 (or x40) microscope objective.



The calibration of the photoactivation

At this step of the experiment, the objective is to provide enough photons to exhaust the conversion of the Actiflash but without generating detrimental side-effects on the biological sample. A calibration process is required to accurately characterize the photon flux generated at the biological sample, in a reproducible way.

In Actiflash conditioned samples (using the concentration previously determined):

- Analyze the phenotype recovery with decreasing illumination duration, starting from 8 minutes to 1 minute.
- Then determine the shortest illumination duration leading to 100% uncaging of Actiflash.

These are three examples of the duration required for uncaging 100% of Actiflash

- **With 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), positioned a few cm above a Petri dish holding the samples: illumination of about 5 minutes is enough to achieve full uncaging of Actiflash.
- **With one-photon excitation in the 350-405 nm wavelength range with laser excitation power P = 10 µW** focused on a spot of diameter 10 µm at the focal plane of the microscope objective (the minimal spot size should be commensurate with the typical size of the cell cross-section): illumination of a few seconds is enough to achieve full uncaging of Actiflash in the few cells along the optical path.
- **With two-photon excitation delivering 200 fs pulses at 750 nm and P = 10 mW:** illumination of 1 second is enough to fully uncage Actiflash in a cell of volume V = 100–1000 µm³.

idylle-labs.com
30, rue Campo Formio
Paris - France
+ 33 1 84 25 51 44
contact@idylle-labs.com

ACT-P 08.01.21