The Twinkle Factory
Stain different, tag FAST.

^{TF}Poppy

Reference 555670-250
Quantity 250 nmol
Store at – 20 °C

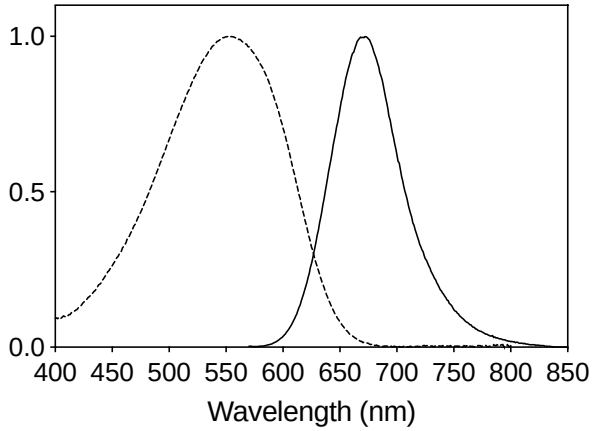
Properties of ^{TF}Poppy when bound to frFAST

Excitation wavelength	555 nm
Emission wavelength	670 nm
Molar absorption coefficient	45,000 $M^{-1}cm^{-1}$
Fluorescence quantum yield	21 %
Affinity constant at 25° C	1 μM

^{TF}Poppy is a membrane-permeant fluorogenic ligand that can be used to selectively label frFAST-tagged proteins in solution, in living cells and in fixed cells. ^{TF}Poppy is almost non-fluorescent when free in solution, but strongly fluoresces when bound to frFAST. ^{TF}Poppy was designed together with a variant of FAST, frFAST, specifically for far-red labeling. It exclusively works with this variant and should not be used with FAST1 nor FAST2. This package includes 250 nmol of ^{TF}Poppy, enabling to prepare 50 mL of a 5 μM labeling solution.

The Twinkle Factory labeling technology is a novel tool that enables the specific fluorescent labeling of any protein of interest. This technology is based on the instantaneous formation of a fluorescent molecular assembly between the small (14 kDa) protein tag FAST and various fluorogenic ligands (^{TF}Fluorogens). ^{TF}Fluorogens strongly fluoresce only when bound to FAST, enabling to detect and image FAST-tagged proteins with high contrast without the need of washing the excess of fluorogenic ligands. The labeling of FAST-tagged proteins with a ^{TF}Fluorogen is non-covalent and can be reversed if necessary by washing.

The use of the Twinkle Factory labeling technology implies cloning and expression of the FAST-tagged protein, and labeling of the resulting fusion with the ^{TF}Fluorogen of choice. The labeling of FAST-tagged proteins is described below. Cells expressing FAST-tagged proteins are not supplied. Note that proteins of interest can be expressed with FAST as either an N- or a C-terminal fusion.



Absorbance (dotted line) and emission (solid line) spectra of ^{TF}Poppy bound to frFAST

Protocol of labeling in living cells

Dissolve one vial of ^{TF}Poppy in 50 μL of DMSO to yield a 5 mM stock solution. Mix by vortexing for few seconds until all the ^{TF}Poppy is dissolved. Note that different stock concentrations can be made depending on your requirements. ^{TF}Poppy is soluble in DMSO up to at least 50 mM.

Dilute the stock solution 1:500 in medium or buffer to yield a 10 μM labeling solution. Mix thoroughly by

vortexing. For best performance, add TFPoppy to serum-free medium or buffer, and do not keep/store the labeling solution. Note that different concentrations can be made depending on your requirements. Optimal concentrations range from 1 to 10 μM.

Remove the cell culture medium, wash with D-PBS, and replace the buffer with the labeling solution. Incubate for 15-30 seconds and image the cells directly.

Image the cells using appropriate settings. frFAST-tagged proteins labeled with TFPoppy have an excitation maximum at 555 nm and an emission maximum at 670 nm.

To reverse the labeling, remove the labeling solution, wash with D-PBS, and replace with culture medium.

Protocol for labeling in fixed cells

Cells expressing FAST-tagged proteins can be fixed before labeling with standard fixation methods such as paraformaldehyde, ethanol, methanol. Once the fixation is performed, wash cells with D-PBS, and replace the buffer with a labeling solution (prepared in D-PBS). Incubate for 15-30 seconds and image the cells directly as above. To reverse the labeling, remove the labeling solution and wash with D-PBS.

Storage

Dry TFPoppy should be stored at – 20 °C in the dark. Once dissolved in DMSO, the solution should be aliquoted to avoid repeated freeze/thaw cycles and stored at – 20 °C in the dark. With proper storage, TFPoppy should be stable at least three years dry or 6 months dissolved in DMSO.

Purity and Characterization

Purity of TFPoppy was determined to be > 99% by nuclear magnetic resonance (NMR) and elementary analysis.

References

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- EP 3 164 411; JP 2017-527,261; WO 2016/001,437; US 10,138,278 (Fluorogen activating and shifting tag (FAST))
- EP 3 404 022; PCT/EP2018/063146; US 2020-0124611 (Membrane-impermeant fluorogenic chromophores)
- EP 3 719 007 (Split photoactive yellow protein complementation system and uses thereof)

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