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⁻¹cm⁻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.

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

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

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vortexing. For best performance, add **Tr**Poppy 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 **Tr**Poppy 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 **Tr**Poppy should be stored at – 20 °C in the dark. With proper storage, **Tr**Poppy should be stable at least three years dry or 6 months dissolved in DMSO.

**Purity and Characterization**

Purity of **Tr**Poppy 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 3 719 007 (Split photoactive yellow protein complementation system and uses thereof)

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