The Twinkle Factory
Stain different, tag FAST.

TFAmber

Reference 499558-250
Quantity 250 nmol
Store at – 20 °C

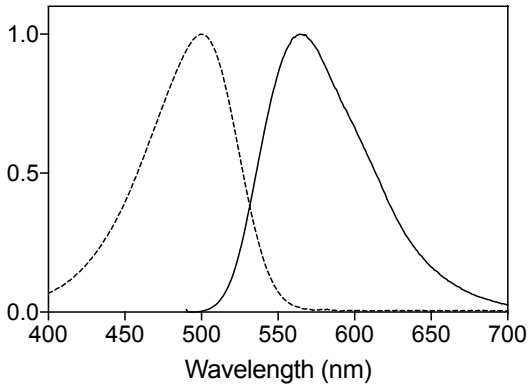
Properties of TFAmber when bound to FAST2

Excitation wavelength	499 nm
Emission wavelength	558 nm
Molar absorption coefficient	46,000 $M^{-1}cm^{-1}$
Fluorescence quantum yield	57 %
Affinity constant at 25° C	0.06 μM

TFAmber is a membrane-permeant fluorogenic ligand that can be used to selectively label FAST-tagged proteins in solution, in living cells and in fixed cells. TFAmber is almost non-fluorescent when free in solution, but strongly fluoresces when bound to FAST1 or FAST2 or their tandem versions tdFAST1 and tdFAST2 (for its properties with FAST1 please visit our website). This package includes 250 nmol of TFAmber, 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 (TFFluorogens). TFFluorogens 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 TFFluorogen is non-covalent and can be reversed if necessary by washing. By using different TFFluorogens, the spectral properties of the FAST-tagged protein can be changed without the need to switch protein tags, providing an experimental versatility not encountered with fluorescent proteins.

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 TFFluorogen 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 TFAmber bound to FAST2

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Protocol of labeling in living cells

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

Dilute the stock solution 1:1000 in medium or buffer to yield a 5 µM labeling solution. Mix thoroughly by vortexing. For best performance, add TFAmber 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. FAST-tagged proteins labeled with TFAmber have an excitation maximum at 499 nm and an emission maximum at 558 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 TFAmber 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, TFAmber should be stable at least three years dry or 6 months dissolved in DMSO.

Purity and Characterization

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

Notice to Buyer/User

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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)

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