The Twinkle Factory
Stain different, tag FAST.

TFCarmine

a.k.a HPAR-3,5DOM
Reference 636715-250
Quantity 250 nmol
Store at 2-8 °C

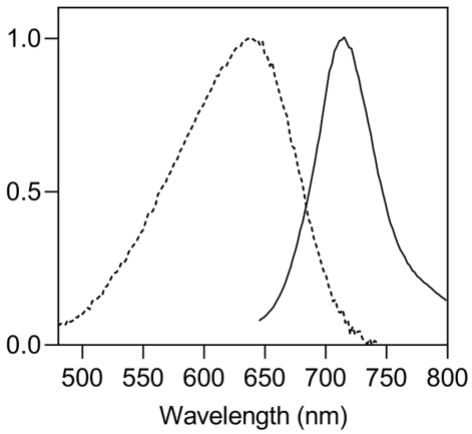
Properties of TFCarmine when bound to nirFAST

Excitation wavelength	636 nm
Emission wavelength	715 nm
Molar absorption coefficient	41 $mM^{-1}cm^{-1}$
Fluorescence quantum yield	7 %
Affinity constant at 25° C	0.036 µM

TFCarmine is a membrane-permeant fluorogenic ligand that can be used to selectively label nirFAST-tagged proteins in solution, in living cells and in fixed cells. TFCarmine is almost non-fluorescent when free in solution, but strongly fluoresces when bound to nirFAST. TFCarmine was designed together with a variant of FAST, nirFAST, specifically for near-infrared labeling. It exclusively works with this variant and should not be used with FAST1 nor FAST2, pFAST, frFAST. Also, it is not recommended to split nirFAST for protein-protein interaction reporting while the affinity of nirFAST for its cognate TFCarmine is very high. This package includes 250 nmol of TFCarmine, enabling to prepare 50 mL of a 5 µM labeling solution.

The Twinkle Factory labeling technology enables the specific fluorescent labeling of any protein of interest. It is based on the instantaneous formation of a fluorescent molecular assembly between the small (14 kDa) protein tag FAST and 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.

The Twinkle Factory labeling technology implies cloning and expressing of the FAST-tagged protein, and labeling 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 by The Twinkle Factory. 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 TFCarmine bound to nirFAST

Protocol of labeling in living cells

Dissolve one vial of TFCarmine in 50 µL of DMSO to yield a 5 mM stock solution. Mix by vortexing for few seconds until

all the TFCarmine is dissolved. Note that different stock concentrations can be made depending on your requirements. TFCarmine 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 TFCarmine 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. nirFAST-tagged proteins labeled with TFCarmine have an excitation maximum at 636 nm and an emission maximum at 715 nm.

To reverse the labeling, remove the labeling solution, wash with D-PBS, and replace with culture medium. Or you can add TFDarth to the culture medium.

Protocol for labeling in fixed cells

Cells expressing nirFAST-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 TFCarmine should be stored at 2-8 °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, TFCarmine should be stable at least three years dry or 6 months dissolved in DMSO.

Purity and Characterization

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

References

bioRxiv 2024-04 (doi.org/10.1101/2024.04.05.588310)

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- EP 3 164 411; JP 2017-527,261; US 10,138,278 (Fluorogen activating and shifting tag (FAST))

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