



The Twinkle Factory

Stain different, tag FAST.

^{TF}Fox-NP

a.k.a HBRAA-3,5DM

Reference 524578-250

Quantity 250 nmol

Store at 2-8 °C

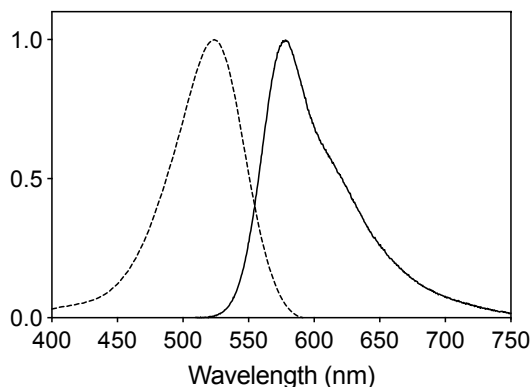
Properties of ^{TF}Fox-NP when bound to pFAST

Excitation wavelength (nm)	524
Emission wavelength (nm)	578
Molar absorption coefficient (M ⁻¹ cm ⁻¹)	58,000
Fluorescence quantum yield (%)	22
Affinity constant at 25° C (μM)	1.8

^{TF}Fox-NP is a membrane-impermeant fluorogenic ligand that can be used to selectively label FAST-tagged proteins at the surface of living cells. ^{TF}Fox-NP is almost non-fluorescent when free in solution, but strongly fluoresces when bound to pFAST. This package includes 250 nmol of ^{TF}Fox-NP, 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. By using different ^{TF}Fluorogens, 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 ^{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}Fox-NP bound to pFAST



The Twinkle Factory

Stain different, tag FAST.

Protocol of labeling in living cells

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

Dilute the stock solution 1:1000 in medium or buffer to yield a 5 μ M labeling solution. Mix thoroughly by vortexing. For best performance, add ^{TF}Fox-NP 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 μ M.

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. pFAST-tagged proteins labeled with ^{TF}Fox-NP have an excitation maximum at 524 nm and an emission maximum at 578 nm.

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

Storage

Dry ^{TF}Fox-NP 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, ^{TF}Fox-NP should be stable at least three years dry or 6 months dissolved in DMSO.

Purity and Characterization

Purity of ^{TF}Fox-NP was determined to be > 99% by nuclear magnetic resonance (NMR) and elementary analysis.

References

Nature Communications **12**, 6989 (2021)

Notice to Buyer/User

The Buyer/User has a non-exclusive license to use this system or any component thereof for research use only. Commercial use of this system or any components thereof requires a license from Twinkle Bioscience S.A.S, 45 rue d'Ulm, 75005 Paris, France. For detailed information, e-mail contact@the-twinkle-factory.com. The products and/or their use may be covered by one or more of the following patents and patent applications:

- EP 3 164 411; JP 2017-527,261; US 10,138,278 (Fluorogen activating and shifting tag (FAST))
- EP 3 404 022; US 2020-0124611 (Membrane-impermeant fluorogenic chromophores)
- EP 3 719 007; US 2022-0169682 (Split photoactive yellow protein complementation system and uses thereof)

These patents and patent applications are owned by CNRS (France), PSL (France), Sorbonne Université (France), and/or Institut Curie (France), and exclusively licensed to Twinkle Bioscience.