



# The Twinkle Factory

Stain different, tag FAST.

## <sup>TF</sup>Lime

Reference P-480541-250  
Quantity 250 nmol  
Store at -20 °C

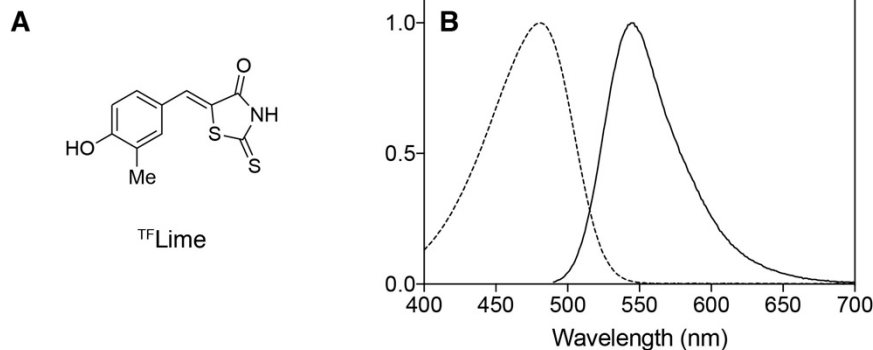
### Properties of <sup>TF</sup>Lime when bound to FAST2

|                              |   |
|------------------------------|---|
| Excitation wavelength        | 480 nm                                  |
| Emission wavelength          | 541 nm                                  |
| Molar absorption coefficient | 41,000 M <sup>-1</sup> cm <sup>-1</sup> |
| Fluorescence quantum yield   | 23 %                                    |
| Affinity constant at 25° C   | 0.07 μM                                 |

<sup>TF</sup>Lime 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. <sup>TF</sup>Lime 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 <sup>TF</sup>Lime, 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 (<sup>TF</sup>Fluorogens). <sup>TF</sup>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 <sup>TF</sup>Fluorogen is non-covalent and can be reversed if necessary by washing. By using different <sup>TF</sup>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 <sup>TF</sup>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.



**(A)** Chemical structure of <sup>TF</sup>Lime. **(B)** Absorbance (dotted line) and emission (solid line) spectra of <sup>TF</sup>Lime bound to FAST2



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### Protocol of labeling in living cells

Dissolve one vial of <sup>TF</sup>Lime in 50  $\mu$ L of DMSO to yield a 5 mM stock solution. Mix by vortexing for few seconds until all the <sup>TF</sup>Lime is dissolved. Note that different stock concentrations can be made depending on your requirements. <sup>TF</sup>Lime is soluble in DMSO up to at least 50 mM.

Dilute the stock solution 1:1000 in medium or buffer to yield a 5  $\mu$ M labeling solution. Mix thoroughly by vortexing. For best performance, add <sup>TF</sup>Lime 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  $\mu$ 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. FAST-tagged proteins labeled with <sup>TF</sup>Lime have an excitation maximum at 480 nm and an emission maximum at 541 nm, and can be imaged with standard GFP settings.

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 <sup>TF</sup>Lime should be stored at  $-20^{\circ}\text{C}$  in the dark. Once dissolved in DMSO, the solution should be aliquoted to avoid repeated freeze/thaw cycles and stored at  $-20^{\circ}\text{C}$  in the dark. With proper storage, <sup>TF</sup>Lime should be stable at least two years dry or 6 months dissolved in DMSO.

### Purity and Characterization

Purity of <sup>TF</sup>Lime was determined to be  $> 99\%$  by nuclear magnetic resonance (NMR) and elementary analysis.

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