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An R&D Systems Company

MATERIAL DATA SHEET

Recombinant 5-TAMRA-Lys Ubiquitin (Ub-Gly-Gly-ε-Lys-TAMRA-Gly-OH) TAMRA

Cat. # U-558

Ubiquitin is a 76 amino acid (aa) protein that is ubiquitously expressed in all eukaryotic organisms. Ubiquitin is highly conserved with 96% aa sequence identity shared between human and yeast Ubiquitin, and 100% aa sequence identity shared between human and mouse Ubiquitin (1). In mammals, four Ubiquitin genes encode for two Ubiquitin-ribosomal fusion proteins and two poly-Ubiquitin proteins. Cleavage of the Ubiquitin precursors by deubiquitinating enzymes gives rise to identical Ubiquitin monomers each with a predicted molecular weight of 8.6 kDa. Conjugation of Ubiquitin to target proteins involves the formation of an isopeptide bond between the C-terminal glycine residue of Ubiquitin and a lysine residue in the target protein. This process of conjugation, referred to as ubiquitination or ubiquitylation, is a multi-step process that requires three enzymes: a Ubiquitin-activating (E1) enzyme, a Ubiquitin-conjugating (E2) enzyme, and a Ubiquitin ligase (E3). Ubiquitination is classically recognized as a mechanism to target proteins for degradation and as a result, Ubiquitin was originally named ATP-dependent Proteolysis Factor 1 (APF-1) (2,3). In addition to protein degradation, ubiquitination has been shown to mediate a variety of biological processes such as signal transduction, endocytosis, and post-endocytic sorting (4-7).

Fluorescence polarization high-throughput screening (HTS) reagent which is based on a 5-Tetramethylrhodamine (TAMRA) modified Lys-Gly sequence that is linked to Ubiquitin via a native isopeptide bond with the lysine side-chain. This reagent is useful for studying Ubiquitin C-terminal hydrolytic activity when detection sensitivity or continuous monitoring of activity at longer wavelengths is essential.

Product Information	
Quantity:	50 µg
Source:	Chemically Synthesized
Stock:	2.3 mg/ml (250 µM) in DMSO
Purity:	>95%, by HPLC. 9163 Da

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Use & Storage

Use: Ubiquitin-Lys-TAMRA is ideal for use as a substrate for Ubiquitin-specific hydrolases. We recommend an assay buffer consisting of 50 mM Hepes pH 7.5, 150 mM NaCl, 2 mM DTT. Fluorescence can be monitored with an excitation wavelength of 544 nm and an emission wavelength of 572 nm. Reaction conditions will need to be optimized for each specific application. We recommend an initial Ubiquitin-Lys-TAMRA concentration of 10-50 nM.

Storage: **Protect from light. Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

Literature

References:

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