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

Ubiquitin substrate

Cat. No. **60-0118-050**
Lot. No. **30114**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 1

Background

A shift in both the excitation and the emission toward longer wavelength helps overcome problems of compound autofluorescence in screening assays. One such substrate is the rhodamine based ubiquitin-εN-(αN-tetramethyl-rhodamine)-lysine (Ubiquitin-Lys-TAMRA), which mimics the naturally occurring isopeptide bond between the C-terminus of ubiquitin and the ε-amino group of a lysine residue of an ubiquitinated protein (Tirat *et al.*, 2005). Cleavage of the isopeptide bond results in a decrease of fluorescence polarization, which makes Ubiquitin-Lys-TAMRA suitable for high-throughput screening applications (Hassiepen *et al.*, 2007). Fluorescence polarization, in contrast to fluorescence intensity, allows a ratiometric read-out of the activity and is thus less sensitive to autofluorescing or quenching caused by test compounds (Tirat *et al.*, 2005).

References:

Hassiepen U, Eidhoff U, Meder G, Bulber JF, Hein A, Bodendorf U, *et al.* (2007) A sensitive fluorescence intensity assay for deubiquitinating proteases using ubiquitin-rhodamine110-glycine as substrate. *Anal Biochem* **371**, 201-207.

Tirat A, Schilb A, Riou V, Leder L, Gerhartz B, Zimmermann J, *et al.* (2005) Synthesis and characterization of fluorescent ubiquitin derivatives as highly sensitive substrates for the deubiquitinating enzymes UCH-L3 and USP-2. *Anal Biochem* **343**, 244-255.

Physical Characteristics

Species: human

Source: synthetic

Quantity: 50 µg

Concentration: 2 mg/ml

Formulation: DMSO

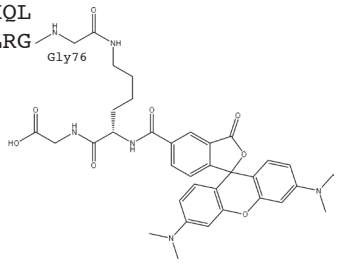
Molecular Weight: 9.16 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:

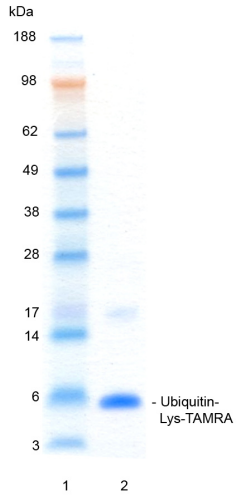
MQIFVKTLTGKTITLEVEPSDTIEN
VKAKIQDKEGIPPDQQRLIFAGKQL
EDGRTLSDYNIQKESTLHLVLRLRG



Ubiquitin (amino acid residues 1-76)
C-terminally tagged
with 5-TAMRA-Lys(Ub)-Gly-OH
Accession number: P62987

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg Ubiquitin-Lys-TAMRA



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The activity of 5-TAMRA-Lys(Ub)-Gly-OH was validated by determining the decrease in mP (measured at Excitation 540, Emission 590) resulting from cleavage of the fluorophore (TAMRA) from Ubiquitin after incubation with UCHL3 (deubiquitylase). UCHL3 was incubated with 5-TAMRA-Lys(Ub)-Gly-OH and fluorescence intensities were measured in the S (parallel) and P (perpendicular) at four time points (0min, 30min, 60min and 90min), from this data mP values were calculated.

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Lot-specific COA version tracker: v1.0.0