

# Ubiquitin-AMC

Ubiquitin substrate



Cat. No. 60-0116-050

Lot. No. 30112

Quantity: 50 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 1

## Background

In addition to fusion proteins, ubiquitin derivatives conjugated with a fluorophore have been reported as substrates for biochemical DUB assays. A frequently used coumarin-based substrate is ubiquitin-7-amido-4-methylcoumarin (Ub-AMC). DUBs catalyze the release of the AMC moiety, which is directly attached to the C-terminus of ubiquitin, and liberation of the fluorophore results in de-quenching of the fluorescent signal (Hassiepen *et al.*, 2007). The excitation/emission range of this fluorophore is 380nm/460nm respectively. The use of this substrate for determining steady-state kinetic parameters in a number of DUB assays was first described by Dang *et al.* (1998).

### References:

Dang LC, Melandri FD and Stein RL (1998) Kinetic and mechanistic studies on the hydrolysis of ubiquitin C-terminal 7-amido-4-methylcoumarin by deubiquitinating enzymes. *Biochemistry* 37, 1868-1879.

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.

## Physical Characteristics

**Species:** human

**Source:** synthetic

**Quantity:** 50 µg

**Concentration:** 2 mg/ml

**Formulation:** DMSO

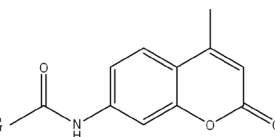
**Molecular Weight:** 8.72 kDa

**Purity:** >98% by InstantBlue™ SDS-PAGE

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

### Protein Sequence:

MQIFVKLTGKTTITLEVEPSDTIEN  
VKAKIQDKEGIPPDQQLIFAGKQL  
EDGRTLSDYNIQKESTLHLVLRRLGG



Ubiquitin (amino acid residues 1-76) C-terminally tagged with AMC (7-amino-4-methylcoumarin)  
Accession number: P62987

## Quality Assurance

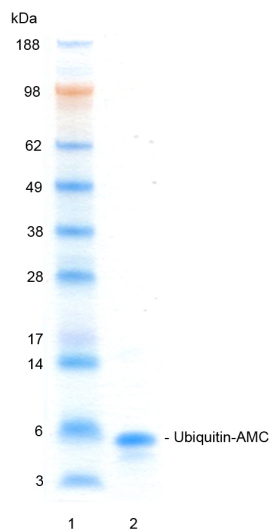
### Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg Ubiquitin-AMC



### Protein Identification:

Confirmed by mass spectrometry.

### Activity Assay:

The activity of Ubiquitin-AMC (7-amido-4-methylcoumarin) was validated by determining the increase in fluorescence at 460nm (Excitation 380nm). Increased fluorescence is a result of the enzyme catalysed cleavage between the C-terminal Glycine and AMC, creating Ubiquitin and de-quenched AMC. UCHL3 (deubiquitylase) was incubated with Ubiquitin-AMC and the fluorescence was measured at four time points (0min, 30min, 60min and 90min).



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Lot-specific COA version tracker: v1.0.0