

Ac-Lys-Gln-Leu-AMC (Ac-KQL-AMC)

Cat. # G3110, G3111

Also Known as: Ac-KQL-AMC; trypsin-like activity substrate of proteasomes

Cas#: N/A

MW (no tag): 586.7 Da

Formula: C₂₉H₄₂N₆O₇

Source: Synthetic

Tag: N/A

Stock Format: Powder

Solubility: Soluble in DMSO up to 50 mM

Concentration: N/A

Quality Assurance: > 92% by HPLC; Enzymatic assay (see data below)

Description: Ac-KQL-AMC is a fluorogenic substrate of the trypsin-like activity of both constitutive and immuno proteasomes. The AMC fluorescence can be detected by a fluorimeter or a plate reader using excitation/emission wavelengths at 360 nm/460 nm, respectively.

This substrate can be used to determine the trypsin-like activity of proteasomes. Working concentration is 50 - 200 μ M.

Storage: Eligible for room temperature shipping. Store at -20°C upon receiving; avoid multiple freeze-thaw cycles after dissolving in DMSO. Protect from light.

Protocol: **Users are strongly recommended to optimize conditions based on their needs.**

1. Briefly spin the product packing tube using a desktop centrifuge to pellet the powder before removing the cap.
2. Prepare a 50 mM substrate stock in DMSO: add 0.17 mL DMSO to 5 mg Ac-KQL-AMC powder or 0.852 mL DMSO to 25 mg Ac-KQL-AMC powder. Vortex to dissolve. Heat in a water bath at 50 °C to dissolve if necessary.
3. Prepare 1X reaction buffer: 20 mM Tris, pH 7.1 at 37 °C, 50 mM NaCl, and 2 mM beta-macaptomethanol.
4. Prepare 2X substrate (200 μ M): add 4 μ l substrate stock prepared in step 2 to 996 μ l warmed (37 °C) 1X reaction buffer. Vortex to dissolve. Keep at 37 °C.
5. Add 50 μ l each sample (using less depending on proteasome amounts in your samples) into a 96 well plate. If less than 50 μ l sample is used, add 1 X reaction buffer to make the sample volume to 50 μ l. Then add 50 μ l 2X substrate prepared in step 4. AMC fluorescence should be recorded immediately (see step 6 blow).
6. The plate reader should be set up for recording AMC fluorescence during the prepreation of the experiment. We recommend to use a 20-30 min kinetic mode to minotor AMC fluorescence . AMC fluorescence can be monitored using excitation/emmission wavelengths at 360 nm/460 nm, respectively.

7. Although proteasomal substrates are preferentially cleaved by proteasomes, other enzymes in cells could cleave them as well. An appropriate control to deduct activities of enzymes other than proteasomes is to include a reaction with 100 μ M MG132 or another proteasome inhibitor. In this reaction, a sample should be preincubated with MG132 for at least 10 min prior to mix with the fluorogenic substrate.

8. The linear slope value (fluorescence unit per min) can be used to represent proteasome activity of each sample. The slope value of the reaction with MG132 should be subtracted from each sample as the background.

Data

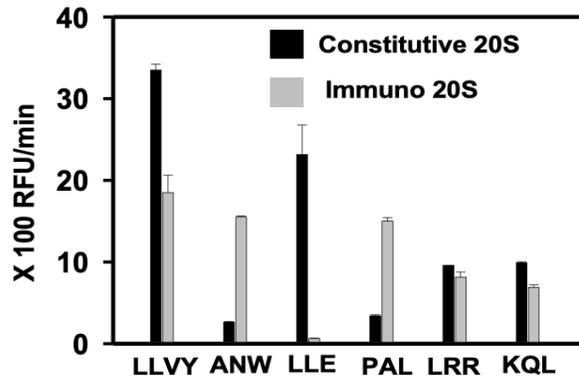


Figure Legend:

20 nM constitutive (catalog # A1400) or immuno (catalog # A1500) bovine 20S proteasome was incubated with 120 nM PA28beta (catalog # A2200) for 15 min in 20 mM Tris, pH 7.1 at 37 °C, 50 mM NaCl, 2 mM β ME . Each substrate was prepared in the same buffer at 100 μ M. Then 50 μ l constitutive or immune 20S proteasome was mixed with 50 μ l each of the substrates into a well of a 96-well plate, and AMC fluorescence was recorded immediately in a 20 min kinetic mode using the exciting/emission filter set at 360/460 nm, respectively. Linear slop of each curve was used to represent the 20S proteasome activity. Values from substrate alone were substracted as background. Error bars represent S.D. of three assays.

References:

1. Blackburn, C., *et al. Biochem. J.* **430**, 461-476 (2010).