Ac-Ala-Asn-Trp-AMC (Ac-ANW-AMC)

Cat. # G1120, G1121

Also Known as:	Ac-ANW-AMC; chymotrypsin-like activity substrate of immunoproteasomes
Cas#:	N/A
MW (no tag):	588.6 Da
Formula:	C30H32N6O7
Source:	Synthetic
Tag:	N/A
Stock Format:	Powder
Solubility:	Soluble in DMSO up to 50 mM
Concentration:	N/A
Quality Assurance:	> 95% by HPLC; Enzymatic assay (see data below)
Description:	Ac-ANW-AMC is a fluorogenic substrate of the chymotrypsin-like activity of proteasomes. This substrate is preferentially cleaved by immunoproteasomes compared to constitutive 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 chymotrypsin-like activity of immunoproteasomes. Working concentration is 50 - 200 µM.
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-ANW-AMC powder or 0.85 mL DMSO to 25 mg Ac-ANW-AMC powder. Vortex to dissolve. Heat in a water bath at 50 0C to dissolve if necessary.
3. Prepare 1X reaction buffer: 20 mM Tris, pH 7.1 at 37 0C, 50 mM NaCl, and 2 mM beta-macaptomethanol.
4. Prepare 2X substrate (200 µM): add 4 µl substrate stock prepared in step 2 to 996 µl warmed (37 0C) 1X reaction buffer. Vortex to dissolve. Keep at 37 0C.
5. Add 50 µl each sample (using less depending on proteasome amounts in your samples) into a 96 well plate. If less than 50 µl sample is used, add 1 X reaction buffer to make the sample volume to 50 µl. Then add 50 µl 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

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 enzyems other than proteasomes is to include a reaction with 100 uM 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

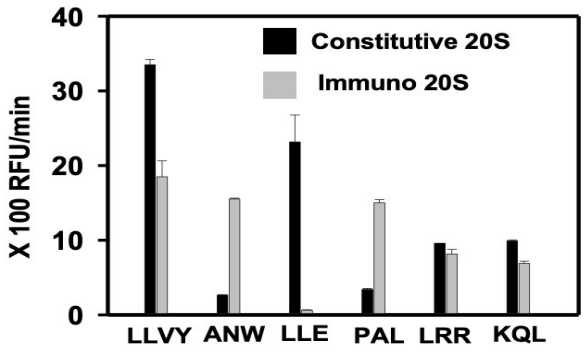

Figure Legend:

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 ^{0}C, 50 mM NaCl, 2 mM βME . Each substrate was prepared in the same buffer at 100 µM. Then 50 µl constitutive or immune 20S proteasome was mixed with 50 µl 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).