

## **Product** Data Sheet

## **Ac-ANW-AMC**

**Cat. No.:** HY-D1705

CAS No.: 2357123-49-8 Molecular Formula:  $C_{30}H_{32}N_6O_7$ 

Molecular Weight: 588.61

Target: Proteasome

Pathway: Metabolic Enzyme/Protease

Storage: Please store the product under the recommended conditions in the Certificate of

Analysis.

## **BIOLOGICAL ACTIVITY**

Biological Activity	
Description	Ac-ANW-AMC is a fluorogenic substrate for immunoproteasome. Ac-ANW-AMC can be used to measure $\beta$ 5i activity (Ex=345 nm, Em=445 nm) $^{[1][2]}$ .
IC <sub>50</sub> & Target	$\beta 5i^{[1]}$
In Vitro	Guidelines (Following is our recommended protocol. This protocol only provides a guideline, and should be modified according to your specific needs) <sup>[2]</sup> .  1. For the proteasome activity assay, cells are lysed in the proteasome activity lysis buffer.  2. Cells are homogenized by passing the lysates 15 times through a 26G×1/2" needle attached to a 1-mL syringe.  3. The lysates are then centrifuged at 12,000 rpm for 15 min. Protein concentrations of the samples are determined using the Bradford assay.  4. Proteasome activity is determined using model peptide substrates by measuring free Ac-ANW-AMC fluorescence on a TECAN infinite m200 fluorometer.  5. The fluorescence unquenched after hydrolysis by proteasomes is monitored every three minutes at 345 nm excitation and 445nm emission wavelengths at 30°C.  Note: Each sample is assayed in triplicate.  MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## **REFERENCES**

[1]. Winter, M.B., La Greca, F., Arastu-Kapur, S., et al. Immunoproteasome functions explained by divergence in cleavage specificity and regulation. eLife 6:e27364, (2017).

[2]. Sumin Kim, et al. Evaluation of Immunoproteasome-Specific Proteolytic Activity Using Fluorogenic Peptide Substrates. Immune Netw. 2022 Apr 15;22(3):e28.

Caution: Product has not been fully validated for medical applications. For research use only.

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