(Ac-WLA)2R110

Catalog number: 13468
Unit size: 1 mg

Component	Storage	Amount
(Ac-WLA)2R110	Freeze (<-15 °C), Minimize light exposure	1 mg

OVERVIEW

The non-fluorescent R110 substrates generate the bright green fluorescent rhodamine 110 product that has Ex/Em = 494/521 nm, and can be easily detected with a FITC filter set. In general, R110 substrates are much more sensitive than the AMC-, AFC- or 4-nitroaniline-based substrates. This R110 substrate is used for monitoring the protease activities of the proteasome. The most common form of the proteasome is known as the 26S proteasome that contains one 20S core particle structure and two 19S regulatory caps. All 20S particles consist of four stacked heptameric ring structures that are themselves composed of two different types of subunits; alpha subunits are structural in nature, whereas beta subunits are predominantly catalytic. The outer two rings in the stack consist of seven alpha subunits each, which serve as docking domains for the regulatory particles and the alpha subunits N-termini form a gate that blocks unregulated access of substrates to the interior cavity. The inner two rings each consist of seven beta subunits and contain the protease active sites that perform the proteolysis reactions. AAT Bioquest offers a group of R110 substrates for monitoring the protease activities of the proteasome at different subsites, i.e., (i) sub-sites: beta1c, Z-LLE-R110; beta2c, Ac-KQL-R110; beta5c, Ac-WLA-R110; beta1i, Ac-PAL-R110; beta2i, Ac-KQL-R110; beta5c, Ac-WLA-R110 and Suc-LLVY-R110; and beta5i, Ac-ANW-R110. The protease activity is measured by monitoring the R110 liberation over time using excitation and emission wavelengths of 490 nm and 520 nm respectively.

AT A GLANCE

Important Following protocol only provides a guideline, and should be modified according to your specific needs.

Product Number	Indicators	Excitation	Emission
13451	(Suc-LLVY)2R110	498 nm	520 nm
13453	Suc-LLVY-AMC	351 nm	430 nm
13455	(Ac-ANW)2R110	498 nm	520 nm
13465	(Ac-KQL)2R110	498 nm	520 nm
13466	(Z-LLE)2R110	498 nm	520 nm
13467	(Ac-PAL)2R110	498 nm	520 nm
13468	(Ac-WLA)2R110	498 nm	520 nm

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. (Ac-WLA)2R110 stock solution (10 mM):
Add 85 µL of DMSO into the vial of 1 mg (Ac-WLA)2R110 to make stock solution (10 mM).

PREPARATION OF WORKING SOLUTION

Proteosome assay solution (2X) (20-40 µM):
Prepare a 20-40 µM of 2X proteasome assay solution in 25 mM HEPES buffer pH 7.5, containing 5mM EDTA, 2mM DTT, and 2% Triton X-100.

SAMPLE EXPERIMENTAL PROTOCOL

1. Mix equal volume of the protesome standards or samples with 2X fluorescent proteasome substrate assay solution, and incubate the solutions at room temperature for at least 1 hour.

2. Monitor the fluorescence using fluorescent microplate readers at 490/525 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

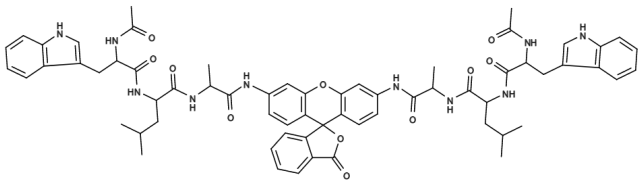

Figure 1. Chemical structure for (Ac-WLA)2R110

DISCLAIMER