Fluorescent Proteasome Substrates

Biological Applications

The main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds. The proteasomal degradation pathway is essential for many cellular processes, including the cell cycle, the regulation of gene expression, and the responses to oxidative stress. The most common form of the proteasome in this pathway is the proteasome 26S, an ATP-dependent proteolytic complex, which contains one 20S (700-kDa) core particle structure and two 19S (700-kDa) regulatory caps. The 20S core contains three major proteolytic activities including chymotrypsin-like, trypsin-like and caspase-like activities. It is responsible for the breakdown of the key proteins involved with apoptosis, DNA repair, endocytosis, and cell cycle control.

AAT Bioquest offers a group of R110 substrates for monitoring the protease activities of the proteasome at different subsites, i.e., (i) sub-sites: β1c, Z-LLE-R110; β2c, Ac-KQL-R110; β5c, Ac-WLA-R110; β1i, Ac-PAL-R110; β2i, Ac-KQL-R110; β5c, Ac-WLA-R110 and Suc-LLVY-R110; and β5i, 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. We also offer Suc-LLVY-AMC, the non-fluorescent substrate generates a bright blue fluorescent AMC product that has Ex/Em = 351/430 nm, and can be easily detected with the DAPI filter set. In general, R110 substrates are much more sensitive than the AMC-, AFC- or 4-nitroaniline-based substrates.

Storage Conditions

Store at < -15 °C and desiccated. Avoid exposure to light. Expiration date is one year upon receipt.

Spectral Properties

Table 1. Spectral Properties of Fluorescent Proteasome Substrates

Product Number	Indicators	Unit	MW	Solvent	Excitation	Emission
13451	(Suc-LLVY)2R110	1 mg	1507.72	DMSO	498 nm	520 nm
13453	Suc-LLVY-AMC	1 mg	763.88	DMSO	351 nm	430 nm
13455	(Ac-ANW)2R110	1 mg	1159.16	DMSO	498 nm	520 nm
13465	(Ac-KQL)2R110	1 mg	1153.33	DMSO	498 nm	520 nm
13466	(Z-LLE)2R110	1 mg	1309.46	DMSO	498 nm	520 nm
13467	(Ac-PAL)2R110	1 mg	977.11	DMSO	498 nm	520 nm
13468	(Ac-WLA)2R110	1 mg	1155.30	DMSO	498 nm	520 nm

Sample Protocol

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

1. Prepare a 5 to 10 mM stock solution in DMSO.
2. Prepare a 2X proteasome substrate (20 to 50 μM) assay solution as the following:

   25 to 50 μL substrate stock solution (10 mM)
   100 μL DTT (1M)
   400 μL EDTA (100 mM)
   10 mL Hepes Buffer (25 mM), pH =7.4

3. Mix equal volume of the protesome standards or samples with 2X fluorescent proteasome substrate assay solution (from Step 1), and incubate the solutions at room temperature for at least 1 hour.
4. Monitor the fluorescence use fluorescent microplate readers.

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