# **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:  $\beta$ 1c, Z-LLE-R110;  $\beta$ 2c, Ac-KQL-R110;  $\beta$ 5c, Ac-WLA-R110;  $\beta$ 1i, Ac-PAL-R110;  $\beta$ 2i, Ac-KQL-R110;  $\beta$ 5c, Ac-WLA-R110 and Suc-LLVY-R110; and  $\beta$ 5i, 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**

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

Table 1. Spectral Properties of Fluorescent Proteasome Substrates

# 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  $\mu$ M) 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.

### **References**

- 1. Wojcik C, Schroeter D, Stoehr M, Wilk S, Paweletz N. (1996) An inhibitor of the chymotrypsin-like activity of the multicatalytic proteinase complex (20S proteasome) induces arrest in G2-phase and metaphase in HeLa cells. Eur J Cell Biol, 70, 172.
- 2. Shi C, Rui Q, Xu LL. (2009) Enzymatic properties of the 20S proteasome in wheat endosperm and its biochemical characteristics after seed imbibition. Plant Biol (Stuttg), 11, 849.
- Sixt SU, Adamzik M, Spyrka D, Saul B, Hakenbeck J, Wohlschlaeger J, Costabel U, Kloss A, Giesebrecht J, Dahlmann B, Peters J. (2009) Alveolar extracellular 20S proteasome in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med, 179, 1098.
- 4. Sollner S, Schober M, Wagner A, Prem A, Lorkova L, Palfey BA, Groll M, Macheroux P. (2009) Quinone reductase acts as a redox switch of the 20S yeast proteasome. EMBO Rep, 10, 65.
- 5. Visekruna A, Joeris T, Schmidt N, Lawrenz M, Ritz JP, Buhr HJ, Steinhoff U. (2009) Comparative expression analysis and characterization of 20S proteasomes in human intestinal tissues: The proteasome pattern as diagnostic tool for IBD patients. Inflamm Bowel Dis, 15, 526.
- 6. Fennell DA, Chacko A, Mutti L. (2008) BCL-2 family regulation by the 20S proteasome inhibitor bortezomib. Oncogene, 27, 1189.
- Majetschak M, Perez M, Sorell LT, Lam J, Maldonado ME, Hoffman RW. (2008) Circulating 20S proteasome levels in patients with mixed connective tissue disease and systemic lupus erythematosus. Clin Vaccine Immunol, 15, 1489.
- 8. Uttenweiler-Joseph S, Claverol S, Sylvius L, Bousquet-Dubouch MP, Burlet-Schiltz O, Monsarrat B. (2008) Toward a full characterization of the human 20S proteasome subunits and their isoforms by a combination of proteomic approaches. Methods Mol Biol, 484, 111.
- 9. Castro-Borges W, Cartwright J, Ashton PD, Braschi S, Guerra Sa R, Rodrigues V, Wilson RA, Curwen RS. (2007) The 20S proteasome of Schistosoma mansoni: a proteomic analysis. Proteomics, 7, 1065.
- 10. Fu Y, Xu B, Zou X, Ma C, Yang X, Mou K, Fu G, Lu Y, Xu P. (2007) Design and synthesis of a novel class of furan-based molecules as potential 20S proteasome inhibitors. Bioorg Med Chem Lett, 17, 1102.

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