Fluorometric Proteasome 20S Activity Assay Kit

Introduction

Proteasomal degradation is one of the main pathways of intracellular protein degradation, which is involved in cellular processes such as apoptosis, gene expression regulation, and oxidative stress, and is closely related to certain cancers, diabetes, and Alzheimer's diseases. Therefore, the proteasome is an important target for drug discovery. The most common proteasome is the proteasome 26S, which contains a 20S core particle structure and two 19S regulatory caps. The 20S core includes three main proteolytic activities, including chymotrypsin-like, trypsin-like, and caspase-like activities.

The Fluorometric Proteasome 20S Activity Assay Kit provides a homogeneous assay for the detection of proteasome 20S activity by the fluorescent substrate LLVY-R110. The proteasome can cleave the LLVY-R110 substrate to produce R110, which emits intensely green fluorescence. Its fluorescence intensity is positively correlated with the activity of the proteasome. The kit is robust enough for high-throughput screening of proteasome inhibitors.

Components and Storage

Components	K2242-100 T
Proteasome LLVY-R110 Substrate	25 μL
Assay Buffer	10 mL

Store the kit at -20°C, stable for 1 year. Proteasome LLVY-R110 Substrate should be stored away from light, and divided into single-use aliquots to avoid repeated freeze/thaw cycles.

Protocol

1. **Preparation of working solution:** Dilute appropriate Proteasome LLVY-R110 Substrate in the Assay Buffer at the dilution ratio of 1:400, and mix well to make the working solution.

***Note:** Allow the Proteasome LLVY-R110 Substrate and Assay Buffer to warm to room temperature. Meanwhile, the Proteasome LLVY-R110 Substrate should be protected from light.

2. **Sample experiment:** Culture cells in suitable plates for a desired period of time. Add an equal volume of working solution in cells. For example, for a 96-well plate containing 100 μL of medium per well, add 100 μL of working solution, or for a 384-well plate containing 25 μL of medium per well, add 25 μL of working solution. Incubate the cells at 37°C away from light for at least 1 h (overnight if needed). The optimal incubation time

varies depending on the cell types.

3. **Detection:** Monitor the fluorescence intensity at Ex/Em = 490/525 nm.

Note

1. The Proteasome LLVY-R110 Substrate should be protected from light when storage and use. And it is recommended to divide into single-use aliquots to avoid repeated freeze/thaw cycles.
2. For your safety and health, please wear lab coats and gloves during the experiment.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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