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Product Information

Proteasome 20S Activity Assay Kit

Catalog Number **MAK172**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Protein degradation occurs most commonly through two major mechanisms, lysosomal degradation and proteasome mediated degradation. Lysosomal degradation occurs through proteolytic enzyme activity and is fairly non-specific. Proteasomal degradation is for a specific targeted protein through the ubiquitination of the target protein. The target protein is typically poly-ubiquitinated and activates the 26S proteasome toward proteolysis. Proteasome 26S is the most common form of the proteasome and is an ATP-dependent proteolytic complex, which contains one 20S (700 kDa) core particle structure and two 19S (700 kDa) regulatory caps.

The proteasome is responsible for the recycling of proteins and maintenance of the balance of protein synthesis and degradation. The proteasome is involved with apoptosis, DNA repair, endocytosis, and cell cycle. It has been implicated in disease states such as certain types of cancer, diabetes mellitus, and Alzheimer's disease among others making it the target for drug discovery investigations.

This Fluorometric Proteasome 20S Assay Kit is a homogeneous fluorescent assay that measures the chymotrypsin-like protease activity associated with the proteasome complex in cultured cells. This kit uses LLVY-R110 as a fluorogenic substrate for proteasome activities. Cleavage of LLVY-R110 by the proteasome generates strongly green fluorescent R110 (λ_{ex} = 480–500 nm/λ_{em} = 520–530 nm). The assay is robust and can be readily adapted for high-throughput assays to evaluate the proteasome activities or screen the inhibitors in cultured cells or in solution. The assay can be performed in a convenient 96 well and 384 well fluorescence multiwell plate formats.

Components

The kit is sufficient for 100 assays in 96 well plates.

Proteosome LLVY-R110 Substrate Catalog Number MAK172A	1 ea
Assay Buffer Catalog Number MAK172B	10 mL
DMSO Catalog Number MAK172C	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well or 384 well flat-bottom plate – It is recommended to use black plates with clear bottoms for best results
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice and storage at –20 °C, protected from light, is recommended.

Preparation Instructions

Allow reagents to come to room temperature and briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Prepare 400× Proteasome LLVY-R110 Substrate Stock Solution by adding 25 µL of DMSO (MAK172C) to the vial of Proteasome LLVY-R110 Substrate (MAK172A) and mixing well.

Prepare Proteasome Assay Loading Solution by adding 25 µL of 400× Proteasome LLVY-R110 Substrate Stock Solution to 10 mL of Assay Buffer (MAK172B) and mixing well.
Notes: 25 µL of 400× Proteasome LLVY-R110 Substrate Stock Solution and 10 mL of Assay Buffer (MAK172B) are enough for 1 plate.

Aliquot and store the unused 400× Proteasome LLVY-R110 Substrate Stock Solution and Assay Buffer at –20 °C.

Procedure
All samples and standards should be run in duplicate.

Cell Preparation
Adherent cells: Plate cells overnight in growth medium at 80,000 cells/well/90 µL for a 96 well plate or 20,000cells/well/20 µL for a 384 well plate.

Non-adherent cells: Centrifuge the cells from the culture medium and then resuspend the cell pellet in culture medium at 300,000 cells/well/90 µL for a 96 well poly-D lysine plate or 80,000 cells/well/20 µL for a 384 well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

Assay Reaction
1. Treat cells with 10 µL of 10× test compound (for a 96 well plate) or 5 µL of 5× test compound (for a 384 well plate) in PBS or desired buffer.

2. Prepare blank well with medium without the cells. Add the corresponding amount of compound buffer as well.

3. Incubate the cell plates in a 5% CO_2 incubator at 37 °C for the desired period of time.

4. Add 100 µL/well (96 well plate) or 25 µL/well (384 well plate) of Proteasome Assay Loading Solution, see Preparation Instructions.
Note: Pure proteasome preparations or cell lysates can be used directly for screening the proteasome inhibitors.

5. Incubate the plate at 37 °C or room temperature for at least 1 hour (2 hours to overnight), protected from light.
Note: Each cell line should be evaluated on an individual basis to determine the optimal incubation time.

6. Monitor the fluorescence intensity at λ_{ex} = 490 nm and λ_{em} = 525 nm.

Results
Calculations
Correct for the background by subtracting the fluorescence of the background blank (medium without cells) from the fluorescence of all test wells.

Comparison of the corrected fluorescence intensity demonstrates relative proteasome 20S activity. Increasing fluorescence intensity indicates increased proteasome 20S activity.

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