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26S Proteasome-mediated Protein Degradation Kit

(Cat. # J4330)

* The supplied materials are sufficient for setting up 32 X 25 ul reactions.

Description

This kit is designed for assaying 26S proteasome-mediated degradation of ubiquitinated proteins in vitro. It contains highly purified active bovine 26S proteasome that has been used by different research groups for assaying protein degradation (see references).

Components

Component	Stock Concentration	Quantity
• Bovine 26S proteasome	200 nM	100 µl
• Epoxomicin	10 mM in DMSO	3 µl
• ATP	500 mM	20 µl
• 10X Degradation Buffer		150 µl

Notes

1. 10X Degradation Buffer: 400 mM Tris, pH 7.1 at 37°C, 400 mM NaCl, 50 mM $MgCl_2$, 20 mM β-ME.
2. The supplied proteasome-specific inhibitor epoxomicin was dissolved in DMSO at 10 mM, dilute 100 X for use.

Protocol

1) In vitro proteasomal degradation is often assayed by a time course assay. In each time point, a 20 ul reaction contains 25 nM purified 26S proteasome, 100 nM polyubiquitinated protein substrate, 4 mM ATP and 10% glycerol in the supplied degradation buffer.

2) We recommend preparing a master reaction mixtures containing the proteasome, ATP, 10% glycerol, buffer and distilled water. For instance, you can prepare a 130 ul master mixtures to set up 5 X 25 ul reactions. In a 130 master mixtures, mix 16.5 µl 26S proteasome, 1.05 µl ATP, 13 µl glycerol (not supplied), 13 µl 10X Degradation buffer and 86.45 µl distilled water. Split the master mixtures into 5 x 25 µl in 0.65 ml Eppendorf tubes. Incubate the mixtures in a 37°C water bath. For the epoxomicin-treated reaction (only necessary to set up one inhibition reaction at the last degradation time point), add 0.25 µl stock epoxomicin in an Eppendorf tube first, then add 25 µl master mixtures. Incubate 10 min at 37°C. To initiate the degradation assay, add 1-2 µl substrate protein to each tube. At each designated time point, add 7 µl 5X SDS sample buffer to

stop the reaction. Make sure your substrate stock concentration is high enough so that you only need to add 1-2 µl in each reaction.

3) The time course and concentrations of the 26S proteasome and a substrate may require optimization.

References

1. Liu CW, Li X, Thompson D, Wooding K, Chang TL, Tang Z, Yu H, Thomas PJ, DeMartino GN. Mol Cell. 2006;24(1):39-50.
2. Jacobson AD, Zhang NY, Xu P, Han KJ, Noone S, Peng J, Liu CW. J Biol Chem. 2009;284(51):35485-94
3. Hemantha HP, Bavikar SN, Herman-Bachinsky Y, Haj-Yahya N, Bondalapati S, Ciechanover A, Brik A. J Am Chem Soc. 2014;136(6):2665-73.