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Ubiquitin-Proteasome Biotechnologies
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26S Proteasome-mediated Protein Degradation Kit

(Cat# J4330)

* The supplied materials are sufficient for setting up 20 X 20 ul reactions.

Description

This kit is designed for assaying 26S proteasome-mediated degradation of polyubiquitinated or non-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	50 µl
• Epoxomicin	10 mM in DMSO	5 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, final working concentration at 10 - 100 µM is sufficient to inhibit the 26S proteasome *in vitro* (100 – 1,000X dilution).

Protocol

1) *In vitro* proteasomal degradation can be assessed by a time course assay. At each time point, a 20 µl reaction contains 25 nM purified 26S proteasome, 50 - 100 nM polyubiquitinated protein or non-ubiquitinated protein (not supplied), 4 mM ATP, and 10% glycerol (not supplied) in the degradation buffer. Time points as well as the proteasome/substrate concentration ratio in the reaction are critical, optimization is often required.
2) We recommend preparing a 2X master reaction mixture containing 50 nM 26S proteasome, 8 mM ATP, 20% glycerol in 1X Degradation Buffer. For instance, you can prepare 60 µl 2X master mixtures to set up 5 X 20 µl reactions. In a microfuge tube, add 26 µl cold water, 6 µl 10X Degradation Buffer, 12 µl glycerol (not supplied), 1 µl ATP stock, mix well by tapping the tube, then add 15 µl proteasome stock, tapping or gently pipetting to mix. Briefly centrifuge at 4 ^{0}C to collect the mixtures if needed. Split the master mixtures into 4 x 10 µl in 0.65 ml microfuge tubes for a time course assay. Keep all tubes on ice.

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3) Incubate the remaining 2X master mixtures in a 37°C water bath for 10 min. To set up the epoxomicin-treated reaction (only set up one inhibition reaction for the last degradation time point), add 0.1 µl epoxomicin stock in a microfuge tube bottom, then add 10 µl warmed 2X master mixtures. If dilution of the original 10 mM epoxomicin stock is desired, prepare an epoxomicin stock in 1X warmed Degradation Buffer first, then mix with 10 µl 2X master reaction mixtures to achieve a final epoxomicin concentration at 10 -100 µM.

4) Incubate all time point tubes including those prepared in STEP 2 at 37 ^{0}C for 10 -15 min. In the meantime, prepare 2X protein substrate stock in 1X Degradation Buffer, and keep at 37 ^{0}C for 5 min to warm. Then add 10 µl 2X substrate protein into each tube to initiate the reaction. For input or the time point 0 reaction, you can add 5 µl 5X SDS sample buffer to 10 µl 2X reaction master mixtures first, then add 10 ul 2X protein substrate.

5) Add 5 µl 5X SDS sample buffer to a reaction mixture at each designated time point (three time points remaining after time 0 and the epoxomicin-treated control). Degradation can be assessed by immunoblotting using an antibody recognizing the protein substrate.

References

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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.