ab139466

Proteasome 26S Degradation Activity Kit

Instructions for Use

For carrying out *in vitro* protein degradation studies with suitably ubiquitinylated protein substrates.

This product is for research use only and is not intended for diagnostic use.

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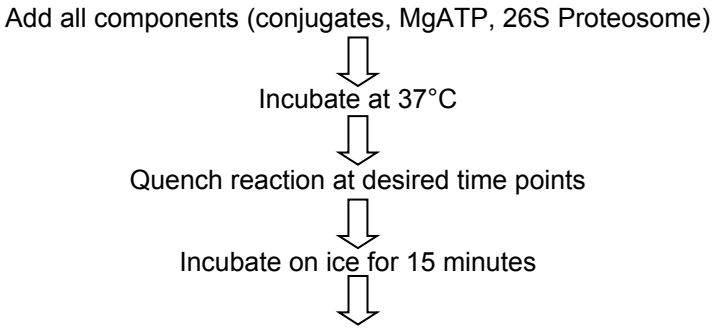
1. Principle of the Assay

Abcam Proteasome 26S Degradation Activity Kit (ab139466) contains a highly purified, Human erythrocyte derived, preparation of 26S proteasomes useful for carrying out *in vitro* protein degradation studies with suitably ubiquitinylated protein substrates. The preparation consists of a high purity mixture of 26S proteasomes singly (26S) and doubly (30S) capped with 19S regulatory subunit complexes in the ratio of 40% single cap: 60% double cap at the time of preparation. Additional kit components include ATP for proteasomal activation.

2. Protocol Summary



3. Materials Supplied

Item	Quantity	Storage
MgATP (2mM)	1 vial	-80°C
26 S Proteosome (5-10nM depending upon conditions)	1 vial	-80°C

4. Storage and Stability

- Store all components -80°C.
- Care must be exercised in the handling and storage of the 26S proteasome to ensure that its activity is not compromised. The complex should be stored at -80°C. When ready for use it should be thawed by standing on ice (i.e. not thawed rapidly!)
- For those wishing to measure and use the enzymatic activity of 26S proteasome it should be used immediately after it is thawed since the enzyme complex is labile after thawing. After dissociation of the complex, the 20S proteasome activity is relatively stable.

5. Materials Required, Not Supplied

- Radio-labelled ubiquitinylated protein conjugate [50-100nM (5000-15000cpm)]
- Scintillation counter
- Pipettes capable of pipetting 1-100 µL accurately.
- 10% trichloroacetic acid (TCA)
- 5% bovine serum albumin.
- Microcentrifuge tubes
- Ice bucket to keep reagents cold
- Water bath or incubator at 37°C
- Centrifuge

6. Assay Protocol

1. Add all components, i.e. conjugates, MgATP, 26S proteasome.
2. Incubate reaction mixture at 37°C for 0-90 minutes.
3. At desired time points, take 10 µL of reaction mixture and add to quenching buffer containing 600 µL of 10% trichloroacetic acid (TCA) and 200 µL 5% bovine serum albumin as a carrier.
4. Vortex and place on ice for 15 minutes.
5. Centrifuge at 4°C for 10 minutes in microcentrifuge at 14000rpm.
6. Count 650 µL of supernatant in scintillation counter.

7. Data Analysis

Plot increase in TCA soluble counts versus time.

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