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.
Table of Contents

1. Principle of the Assay 3
2. Protocol Summary 3
3. Materials Supplied 4
4. Storage and Stability 4
5. Materials Required, Not Supplied 5
6. Assay Protocol 6
7. Data Analysis 6
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 ubiquitylated 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

Add all components (conjugates, MgATP, 26S Proteosome) → Incubate at 37°C → Quench reaction at desired time points → Incubate on ice for 15 minutes → Centrifuge and measure in scintillation counter
3. Materials Supplied

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgATP (2mM)</td>
<td>1 vial</td>
<td>-80°C</td>
</tr>
<tr>
<td>26 S Proteosome (5-10nM depending upon conditions)</td>
<td>1 vial</td>
<td>-80°C</td>
</tr>
</tbody>
</table>

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 \( \mu L \) of reaction mixture and add to quenching buffer containing 600 \( \mu L \) of 10\% trichloroacetic acid (TCA) and 200 \( \mu L \) 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 \( \mu L \) of supernatant in scintillation counter.

7. Data Analysis

Plot increase in TCA soluble counts versus time.
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

Copyright © 2013 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.