ab213974
Proteasome ELISA kit

For the quantitative determination of 20S Proteasome in biological samples.

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

The Proteasome ELISA kit (ab213974) is intended for the quantitative determination of 20S Proteasome concentrations in biological samples using a sandwich ELISA technique, utilizing two 20S proteasome specific antibodies for capture and detection purposes together with a highly sensitive substrate. Sample 20S proteasome levels are determined by comparison to a 20S proteasome calibration curve produced in parallel. This kit provides sufficient material for 1 x 96 well plate set-up to be run.

Proteasomes are non-lysosomal proteolytic complexes localized primarily in the cytoplasm and in the nucleus of eukaryotic cells. The 26S proteasome structure is composed of a 20S proteasome catalytic core complex and one or two 19S regulatory sub complexes. The 20S core comprises two copies of 14 subunits (7 α–subunits and 7 β–subunits) arranged in a α7β7β7α7 cylindrical array, varying catalytic subunit composition (β1, β1i; β2, β2i; β5, β5i) results in a variety of possible subtypes. The 19S regulatory sub complexes, comprised of 6 ATPase and at least 10 non-ATPase subunits, specifically bind ubiquitinylated proteins and provide the 20S core with ATP-ubiquitin–dependent proteolytic activity.

The ubiquitin-proteasome system is the major non-lysosomal system for the degradation of short half-life proteins and peptides that are involved in basic cellular processes, such as cell-cycle regulation and apoptosis, transcriptional regulation, or antigen processing. Thus, protein degradation by the ubiquitin-proteasome pathway has a major regulatory function for proliferation activity and survival of both normal and malignant cells. The 20S proteasome has been detected in normal human blood plasma (known as circulating proteasome), possessing comparatively low specific activity and with a distinct pattern of subtypes.

Proteasomes are often overexpressed in cancer cells; abnormally high expression of proteasomes having been found in human leukemia cells, renal cancer cells and in breast cancer cell lines. In patients suffering from auto-immune diseases, malignant myelo-proliferative syndromes, multiple myeloma, acute and chronic lymphatic leukemia, solid tumor, sepsis or trauma, the concentration of circulating proteasome has been found to be elevated correlating with the disease state, and may have prognostic significance.
Proteasome levels have been measured by enzyme-linked immunosorbent assay (ELISA) techniques in cell lysates, serum or plasma samples. This approach has been used to show that proteasome concentrations in peripheral blood are elevated in patients with certain types of malignant diseases, including multiple myeloma, suggesting that circulating proteasome levels may be correlated with tumor burden. The link between elevated circulating proteasome levels and disease activity has also been demonstrated in patients with systemic autoimmune diseases.
2. Protocol Summary

Add diluted Proteasome Antibody into each well and incubate overnight (+2-8°C)

↓

Wash plate

↓

Add Blocking Buffer to wells and incubate (RT)

↓

Wash plate

↓

Add Standards and Samples to wells and incubate (RT)

↓

Wash plate

↓

Add Detection antibody to wells and incubate (RT)

↓

Wash plate

↓

Add Goat anti-Rabbit:HRP antibody and incubate (RT)

↓

Wash plate

↓

Add TMB substrate solution

↓

Add Stop solution and read plate at 450 nm
3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All ELISA kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never Pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store ELISA kit at -20°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.
5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage Condition (Before prep)</th>
<th>Storage Condition (After prep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteasome coated microplate (12x 8 well strips)</td>
<td>96 wells</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Proteasome Antibody</td>
<td>2 x 25 μL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Proteasome Detection Antibody</td>
<td>25 μL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Goat anti-Rabbit:HRP Antibody</td>
<td>300 μL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Proteasome Stock Solution (0.4mg/mL)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Binding Buffer 50X</td>
<td>500 μL</td>
<td>-20°C</td>
<td>RT</td>
</tr>
<tr>
<td>ELISA Buffer 1X</td>
<td>100 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Lysis Buffer 5X</td>
<td>5 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Wash Buffer Concentrate</td>
<td>100 mL</td>
<td>-20°C</td>
<td>RT</td>
</tr>
<tr>
<td>Blocking Buffer 1X</td>
<td>30 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>10 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>10 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3 units</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
7. Materials Required, Not Supplied

These materials are not included in the ELISA kit, but will be required to successfully perform this assay:
- Deionized or distilled water
- Precision Pipettes for volumes between 100 μL and 1,000 μL
- Repeater Pipette for dispensing 100 μL
- Disposable beakers for diluting buffer concentrates
- Graduated cylinders
- Glass or plastic tubes for diluting and aliquoting standard
- A microplate shaker
- Absorbent paper for blotting
- Microplate reader capable of reading at 450nm, preferably with correction between 570 nm and 590 nm
- Graph paper for plotting the standard curve
8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- When generating positive control samples, it is advisable to change Pipette tips after each step.
- This ELISA kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Proteasome coated microplate (12x 8 well strips):
A 96 well flat-bottomed plate using break-apart strips. Ready to use. Store at -20°C.

9.2 Proteasome Antibody:
Alpha 6 subunit, 20S proteasome, mouse monoclonal antibody. 2 x 25 μL. Store stock at -20°C. Dilute the desired amount of Proteasome Antibody to a concentration of 1:500 in 1X Binding Buffer (100 μL per well). 24 μL into 12 mL is required for a full 96-well plate.

9.3 Proteasome Detection Antibody:
25 μL. Store stock at -20°C. Dilute the desired amount of Proteasome Detection Antibody to a concentration of 1:1,000 in ELISA Buffer (100 μL required per well). 12 μL into 12 mL is required for a full 96-well plate.

9.4 Goat anti-Rabbit:HRP Antibody:
300 μL. Store stock at -20°C. Dilute the desired amount of Goat anti-Rabbit:HRP Antibody to a concentration of 1:100 in ELISA Buffer (100 μL per well). 120 μL into 12 mL is required for a full 96 well plate.

ΔNote: Highly diluted antibodies are not stable and should not be stored! Prepare fresh dilutions as required.

9.5 Proteasome Stock Solution:
(0.4 mg/mL)1 vial. Store stock at -20°C.
9.6 **Binding Buffer 50X:**
500 µL. Store stock at -20°C. Prepare the desired amount of 1X Binding Buffer by diluting the 50X concentrate 1:50 in deionized water (100 µL per well). To make 50 mL 1X Binding Buffer, add 1 mL 50X Binding Buffer to 49 mL deionized water. This can be stored at room temperature until the kit’s expiration, or for 3 months, whichever is earlier.

9.7 **ELISA Buffer 1X:**
100 mL. Ready to use. Store at -20°C.

9.8 **Lysis Buffer 5X:**
5 mL. Store stock at -20°C. Allow to come to room temperature. Prepare the desired amount of 1X Lysis Buffer by diluting the 5X concentrate 1:5 in deionized water. Ensure buffer is completely in solution prior to use. To make 10 mL, add 2 mL 5X Lysis Buffer to 8 mL deionized water.

9.9 **Wash Buffer Concentrate:**
100 mL. Store stock at -20°C. Prepare 1X Wash Buffer by diluting 50 mL of the supplied Wash Buffer Concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit’s expiration, or for 3 months, whichever is earlier.

9.10 **Blocking Buffer 1X:**
30 mL. Ready to use. Store at -20°C.

9.11 **TMB Substrate:**
10 mL. Ready to use. Store at -20°C.

9.12 **Stop Solution:**
10 mL. Ready to use. Store at -20°C.

9.13 **Plate Sealer:**
3 units. Ready to use. Store at -20°C.
10. Standard Preparation

10.1 Dilute the desired amount of Proteasome Stock Solution (0.4 mg/mL) to a concentration of 1:250 by adding 4 µL to 996 µL ELISA Buffer then serially dilute 1:2 to give Proteasome dilutions of 1.6 µg/mL, 0.8 µg/mL, 0.4 µg/mL, 0.2 µg/mL, 0.1 µg/mL, 0.05 µg/mL, 0.025 µg/mL, and 0 µg/mL (S0).

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Standard (µL)</th>
<th>ELISA Buffer (µL)</th>
<th>Final volume (µL)</th>
<th>End Conc. (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 Stock</td>
<td>996</td>
<td>500</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>500 Std. 1</td>
<td>500</td>
<td>500</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>500 Std. 2</td>
<td>500</td>
<td>500</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>500 Std. 3</td>
<td>500</td>
<td>500</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>500 Std. 4</td>
<td>500</td>
<td>500</td>
<td>0.1</td>
</tr>
<tr>
<td>6</td>
<td>500 Std. 5</td>
<td>500</td>
<td>500</td>
<td>0.05</td>
</tr>
<tr>
<td>7</td>
<td>500 Std. 6</td>
<td>500</td>
<td>500</td>
<td>0.025</td>
</tr>
<tr>
<td>8</td>
<td>N/A</td>
<td>500</td>
<td>500</td>
<td>0</td>
</tr>
</tbody>
</table>

![Diagram showing dilution process]
11. Sample Preparation

- The assay is suitable for the measurement of proteasome in plasma, serum and cell lysate samples.
- Plasma/serum samples should be diluted between 1:2 and 1:50 of their original concentration and cell lysate samples between 1:100 and 1:5000 of their original concentration in ELISA Buffer prior to use. This dilution may require optimization to give results within the detection limit of the proteasome ELISA kit (1.6 μg/mL).

- Cell lysate preparation method (if required):
  11.1 Wash cells with PBS
  11.2 Lyse them in Lysis Buffer (diluted to 1X) at a concentration of approximately 1×10⁷ cells/mL.
  11.3 Vortex the lysate briefly and incubate for 15 minutes at +2-8°C (the lysate preparation can be stored at -20°C at this point).
  11.4 Spin at 10,000 rpm for 5 minutes and transfer the supernatant to a new tube.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at +2-8°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.
13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

13.1 To prepare plate, Pipette 100 µL diluted 1:500 Proteasome Antibody into each ELISA plate well. Cover plate with plastic wrap and incubate overnight at +2-8°C.

13.2 Discard the solution in the wells and add 300 µL 1X Wash Buffer using a multichannel Pipette. Repeat for a total of 5 washes, removing all liquid between washes.

13.3 Block plate with addition of 300 µL Blocking Buffer to each well. Place plate on rocker and incubate for 2 hours at room temperature.

13.4 Repeat plate washing as above.

13.5 Pipette 100 µL of ELISA Buffer into the S0 (0 µg/mL standard) wells except Blank.

13.6 Pipette 100 µL of Proteasome dilutions #1 through #7 into the appropriate wells, except the Blank.

13.7 Pipette 100 µL of the Samples into the appropriate wells.

13.8 Tap the plate gently to mix the contents.

13.9 Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.

13.10 Empty the contents of the wells and wash by adding 300 µL of 1X Wash Buffer to every well. Repeat the wash 4 more times for a total of 5 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.11 Pipette 100 µL of 1:1,000 Proteasome Detection Antibody into each well, except the Blank.

13.12 Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.

13.13 Empty the contents of the wells and wash by adding 300 µL of 1X Wash Buffer to every well. Repeat the wash 4 more times for a total of 5 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.14 Add 100 µL of 1:100 Goat anti-Rabbit:HRP Antibody to each well, including the Blank.
13.15 Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.

13.16 Empty the contents of the wells and wash by adding 300 µL of 1X Wash Buffer to every well. Repeat the wash 4 more times for a total of 5 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.17 Pipette 100 µL of TMB Substrate into each well.

13.18 Incubate for 30 minutes at room temperature on a plate shaker at ~500 rpm.

13.19 Pipette 100 µL Stop Solution to each well.

13.20 Read the optical density at 450 nm, preferably with correction between 570 and 590 nm.
14. Calculations

If data reduction software is not readily available, the concentration of 20S proteasome can be calculated as follows:

14.1 Calculate the average net Optical Density (OD) for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

\[ \text{Average Net OD} = \text{Average OD} - \text{Average Blank OD} \]

14.2 Using linear graph paper, plot the Average Net OD for each standard versus 20S proteasome concentration in each standard. Approximate a straight line through the points. The concentration of 20S proteasome in the unknowns can be determined by interpolation.
15. Typical data

**Typical standard** curve – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average OD</th>
<th>Net OD</th>
<th>20S Proteasome (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>(0.175)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>S0</td>
<td>0.175</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S1</td>
<td>1.54</td>
<td>1.365</td>
<td>1.6</td>
</tr>
<tr>
<td>S2</td>
<td>0.921</td>
<td>0.745</td>
<td>0.8</td>
</tr>
<tr>
<td>S3</td>
<td>0.562</td>
<td>0.387</td>
<td>0.4</td>
</tr>
<tr>
<td>S4</td>
<td>0.385</td>
<td>0.210</td>
<td>0.2</td>
</tr>
<tr>
<td>S5</td>
<td>0.293</td>
<td>0.117</td>
<td>0.1</td>
</tr>
<tr>
<td>S6</td>
<td>0.234</td>
<td>0.058</td>
<td>0.05</td>
</tr>
<tr>
<td>S7</td>
<td>0.217</td>
<td>0.041</td>
<td>0.025</td>
</tr>
</tbody>
</table>

![Figure 1. Proteasome ELISA Kit (ab213974) Standard Curve](image)
<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Inaccurate Pipetting</td>
<td>Check Pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standard dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
</tr>
<tr>
<td>Low Signal</td>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times standard/sample incubation</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check Pipettes and ensure correct preparation</td>
</tr>
<tr>
<td></td>
<td>Incubation times with TMB too brief</td>
<td>Ensure sufficient incubation time until blue color develops prior addition of Stop solution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the ELISA kit</td>
<td>All components 4°C. Keep TMB substrate solution protected from light.</td>
</tr>
</tbody>
</table>

16. Troubleshooting
17. Notes
Technical Support

Copyright © 2016 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259

France
supportscientifique@abcam.com | 01.46.94.62.96

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

Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com

UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
can.technical@abcam.com | 877-749-8807

US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823

China
cn.technical@abcam.com | +86-21-5110-5938 | 400-628-6880

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

Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450

New Zealand
nz.technical@abcam.com | +64-(0)9-909-7829