ELISA PRODUCT INFORMATION & MANUAL

20S Proteasome

NBP2-62173

Enzyme-linked Immunosorbent Assay for quantitative detection of Human 20S Proteasome.

For research use only.

Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Novus Biologicals Technical Support if necessary.

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DESCRIPTION

This kit provides the means to quantify 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 × 96 well plate set-up to be run.

INTRODUCTION

Proteasomes are non-lysosomal proteolytic complexes localized primarily in the cytoplasm and in the nucleus of eukaryotic cells[1]. 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[2].

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[3,4]. Thus, protein degradation by the ubiquitin-proteasome pathway has a major regulatory function for proliferation activity and survival of both normal and malignant cells[5,6]. 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[7].

Proteasomes are often overexpressed in cancer cells; abnormally high expression of proteasomes having been found in human leukemia cells[8], renal cancer cells[9] and in breast cancer cell lines[10]. 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[7].

Proteasome levels have been measured by enzyme-linked immunosorbent assay (ELISA) techniques in cell lysates, serum or plasma samples[11,12]. This approach has been used to show that proteasome concentrations in peripheral blood are elevated in patients with certain types of malignant diseases[13,14,15], including multiple myeloma[11], suggesting that circulating proteasome levels may be correlated with tumor burden[14,15]. The link between elevated circulating proteasome levels and disease activity has also been demonstrated in patients with systemic autoimmune diseases[16].

SAFETY WARNINGS & PRECAUTIONS

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Handle with care

Avoid freeze / thaw cycles

- Stop Solution 2 is a 1N hydrochloric acid solution. This solution is caustic; care should be taken in use.
- The activity of the Goat anti-Rabbit:HRP Antibody is affected by nucleophiles such as azide, cyanide and hydroxylamine.
- We test this kit's performance with a variety of buffers, however it is possible that high levels of interfering substances may cause variation in assay results.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. All biological materials should be disposed of in accordance with established safety procedures.

MATERIALS SUPPLIED

1. **Microtiter Plate: one 96 well plate**

   A 96 well flat-bottomed plate using break-apart strips

2. **Proteasome 20S alpha6 subunit, mAb: 2 x 25 µL**

   Alpha6 subunit, 20S proteasome, mouse monoclonal antibody

3. **20S Proteasome Detection Antibody: 1 x 25 µL,**

   20S proteasome, rabbit polyclonal antibody

4. **Goat anti-Rabbit:HRP Antibody: 1 x 300 µL**

   Goat anti-rabbit IgG conjugated to Horseradish peroxidase

5. **20S Proteasome Stock Solution: 1 x 4 µg**

   4 µg (0.4 mg/mL) of Proteasome 20S.

6. **Binding Buffer, 50X: 1 x 0.5 mL**

   Phosphate buffered saline

7. **ELISA Buffer, 1X: 1 x 100 mL**

   Tris buffered saline containing BSA and detergents

8. **Lysis Buffer, 5X: 1 x 5 mL**

   100 mM Tris HCl, pH 7.4, 750 mM NaCl, 10 mM EDTA, 2.5% NP-40.

9. **Wash Buffer Concentrate: 1 x 100 mL**

   Tris buffered saline containing detergents.

10. **Blocking Buffer, 1X: 1 x 30 mL**

    Phosphate buffered saline containing protein

11. **TMB Substrate: 1 x 10 mL**

    A solution of 3,3’,5,5’ tetramethylbenzidine (TMB) and hydrogen peroxide. Protect from prolonged exposure to light.

12. **Stop Solution 2: 1 x 10 mL**

    A 1 N solution of hydrochloric acid in water. Keep tightly capped. **Caution: Caustic.**

13. **Plate Sealer, 3 each**

Reagants require separate storage conditions.

STORAGE

Upon receipt store the full kit at -20°C until required. Avoid freeze/thaw cycles.

OTHER MATERIALS NEEDED

1. Deionized or distilled water
2. Precision pipets for volumes between 100 µL and 1,000 µL
3. Repeater pipet for dispensing 100 µL
4. Disposable beakers for diluting buffer concentrates
5. Graduated cylinders
6. Glass or plastic tubes for diluting and aliquoting standard
7. A microplate shaker
8. Adsorbent paper for blotting
9. Microplate reader capable of reading at 450nm, preferably with correction between 570nm and 590nm
10. Graph paper for plotting the standard curve

SAMPLE HANDLING

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):

1. Wash cells with PBS
2. Lyse them in Lysis Buffer (diluted to 1X) at a concentration of approximately 1×10^7 cells/mL.
3. Vortex the lysate briefly and incubate for 15 minutes at 4°C (the lysate preparation can be stored at -20°C at this point).
4. Spin at 10,000 rpm for 5 minutes and transfer the supernatant to a new tube.

PROCEDURAL NOTES

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards must be made up in polypropylene tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. Reagents with a volume less than 100 µL should be centrifuged.
8. This kit uses break-apart microtiter strips, which allows the user to measure as many samples as desired. However, we do suggest that for optimal results the wells should be used in the assay while still wet, immediately following the plate coating procedure.
9. Prior to addition of antibody, conjugate and substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.
10. Once reagents have been added to the 96-well ELISA plate, DO NOT LET IT DRY OUT at any time during the assay.

REAGENT PREPARATION

1. **Wash Buffer, 1X**

   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.

2. **Binding Buffer, 1X**

   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.

3. **Proteasome 20S alpha6 subunit, mAb**

   Dilute the desired amount of Proteasome 20S alpha 6 subunit monoclonal 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.

4. **20S Proteasome Detection Antibody**

   Dilute the desired amount of 20S 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.

5. **Goat anti-Rabbit:HRP Antibody**

   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.

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

6. **20S Proteasome Stock Solution**

   Dilute the desired amount of 20S Proteasome Stock Solution (0.4mg/mL) to a concentration of 1:250 in ELISA Buffer. Add 4 µL to 996 µL ELISA Buffer and serially dilute 1:2 to give 20S 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).

Reconstituted and diluted standards should be used within 60 minutes of preparation.

Discard any unused reconstituted standard and subsequent dilutions.

7. Cell Lysis Buffer, 1X

Allow to come to room temperature. Prepare the desired amount of 1X Lysis Buffer by diluting the 5X concentrate (80-KW0865-0005) 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.

ASSAY PROCEDURE

Bring all reagents to room temperature for at least 30 minutes prior to opening.

1. To prepare plate, pipet 100 µL diluted 1:500 Proteasome 20S alpha6 subunit monoclonal Antibody into each ELISA plate well. Cover plate with plastic wrap and incubate overnight at 4°C.
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.
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.
4. Repeat plate washing as above.
5. For most optimal results, it is best to use the plate wet immediately following the plate coating procedure.

All standards and samples should be run in duplicate.

6. Pipet 100 µL of ELISA Buffer into the S0 (0 µg/mL standard) wells except Blank.
7. Pipet 100 µL of 20S Proteasome dilutions #1 through #7 into the appropriate wells, except the Blank.
8. Pipet 100 µL of the Samples into the appropriate wells.
9. Tap the plate gently to mix the contents.
10. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500rpm.
11. Empty the contents of the wells and wash by adding 400 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.
12. Pipet 100 µL of 1:1,000 20S Proteasome Detection Antibody into each well, except the Blank.
13. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500rpm.
14. Empty the contents of the wells and wash by adding 400 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.
15. Add 100 µL of 1:100 Goat anti-Rabbit:HRP Antibody to each well, including the Blank.
16. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500rpm.

17. Empty the contents of the wells and wash by adding 400 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.
18. Pipet 100 µL of TMB Substrate into each well.
19. Incubate for 30 minutes at room temperature on a plate shaker at ~500rpm.
20. Pipet 100 µL Stop Solution 2 to each well.
21. Read the optical density at 450nm, preferably with correction between 570 and 590nm.

CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of 20S proteasome in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of 20S proteasome can be calculated as follows:

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

   Average Net OD = Average OD - Average Blank OD

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.

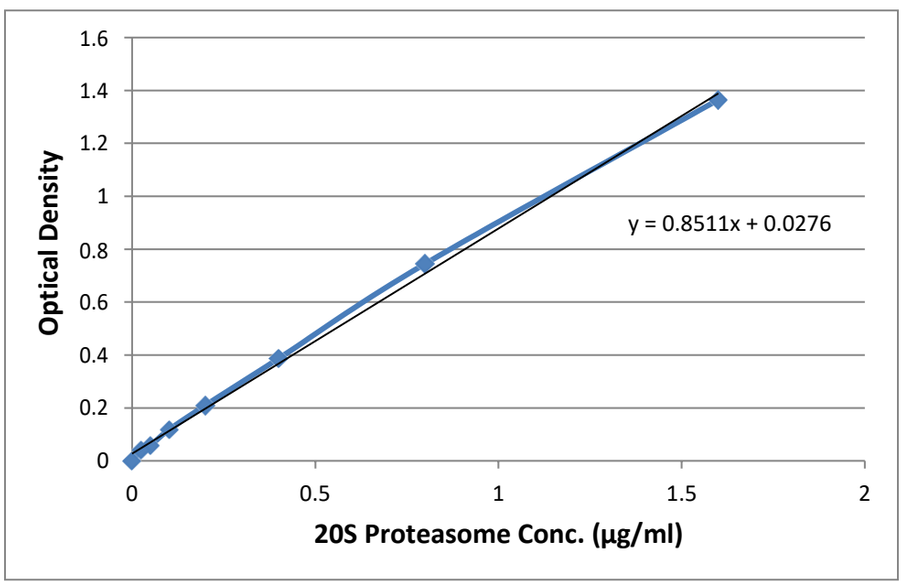
TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results.

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

TYPICAL STANDARD CURVES

A typical standard curve is shown below. This curve must not be used to calculate 20S proteasome concentrations; each user must run a standard curve for each assay.



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