Product Manual

Human Beta 2 Microglobulin ELISA Kit

Catalog Number

PRB- 5038 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Beta 2 Microglobulin (ß2M) is a protein component of the MHC Class I complex. ß2M is a peripheral membrane protein rather than a transmembrane protein, associating closely to the MHC Class I α3 transmembrane chain on the cell surface of all cells containing a nucleus. ß2M is required for stabilizing the peptide binding groove as well as ensuring cell surface expression/localization of MHC Class I. In addition to MHC Class I, ß2M can associate with similar structure proteins like Qa and CD1. In many tissue types, the mRNA levels of ß2M are of intermediate level which has lead to increased use of this gene target for normalization in quantitative reverse transcription PCR (qRT-PCR). While the normal level of ß2M protein in human serum is <2 µg/mL, in cases of lymphoma or multiple myeloma it can be measurably higher. In multiple myeloma, the protein level of ß2M in the blood has become an important prognostic indicator, with a level less than 4 µg/mL correlating with a median survival time of 43 months, while greater than 4 µg/mL concentration can expect median survival of 12 months.

Cell Biolabs' Human Beta 2 Microglobulin ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human ß2M in plasma, serum, cell or tissue lysate samples. The kit has a detection sensitivity limit of 300 pg/mL human ß2M. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. PRB-5033: Human Alpha 2 Macroglobulin ELISA Kit
2. PRB-5034: Human Alpha 1 Antitrypsin ELISA Kit
3. CBA-220: CytoSelect™ 96-Well Phagocytosis Assay, Red Blood Cell Substrate
4. CBA-224: CytoSelect™ 96-Well Phagocytosis Assay, Zymosan Substrate
5. CBA-250: CytoSelect™ Cell Proliferation Assay Reagent (Fluorometric)
6. CBA-251: CytoSelect™ BrdU Cell Proliferation ELISA Kit
7. CBA-252: CytoSelect™ MTT Cell Proliferation Assay
8. CBA-253: CytoSelect™ WST-1 Cell Proliferation Assay Reagent

Kit Components

Box 1 (shipped at room temperature)

1. Anti-ß2M Antibody Coated Plate (Part No. 50381B): One 96-well strip plate (8 x 12).
2. Anti-Human ß2M Antibody-HRP Conjugate (1000X) (Part No. 50382C): One 10 µL vial.
3. Assay Diluent (Part No. 310804): One 50 mL bottle.
4. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
5. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
6. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human ß2M Standard (Part No. 50383D): One 30 µL vial of 2 µg/mL Human ß2M.

Materials Not Supplied

1. Plasma, serum, cell or tissue lysate
2. PBS containing 0.1% BSA
3. PBS containing 1% NP40
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Human ß2M Standard at -80ºC and the Anti-Human ß2M Antibody-HRP conjugate at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Human ß2M Antibody HRP Conjugate: Immediately before use dilute the Anti-Human ß2M Antibody HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human ß2M Standard

Prepare a dilution series of human ß2M standards in the concentration range of 0 to 20 ng/mL into Assay Diluent (Table 1).

Standard Tubes	2 µg/mL Human ß2M Standard (µL)	Assay Diluent (µL)	Human ß2M (ng/mL)
1	5	495	20
2	250 of Tube #1	250	10
3	250 of Tube #2	250	5
4	250 of Tube #3	250	2.5
5	250 of Tube #4	250	1.25
6	250 of Tube #5	250	0.625
7	250 of Tube #6	250	0.313
8	0	250	0

Table 1. Preparation of Human ß2M Standards

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Normal plasma samples require 1:50-1:400 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Assay immediately or store samples at -80ºC for up to three months. Normal serum samples require 1:50-1:400 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Urine: Harvest urine and centrifuge for 10 minutes at 1000 x g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold PBS containing 1% NP40 and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 1% NP40 and 0.1% BSA as needed.

Assay Protocol

1. Add 100 µL of human ß2M unknown sample or standard to the Anti-Human ß2M Antibody Coated Plate. Each human ß2M unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at room temperature for 1 hour on an orbital shaker.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Anti-Human ß2M Antibody HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
6. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

7. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
8. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Human Alpha 1 Antitrypsin ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

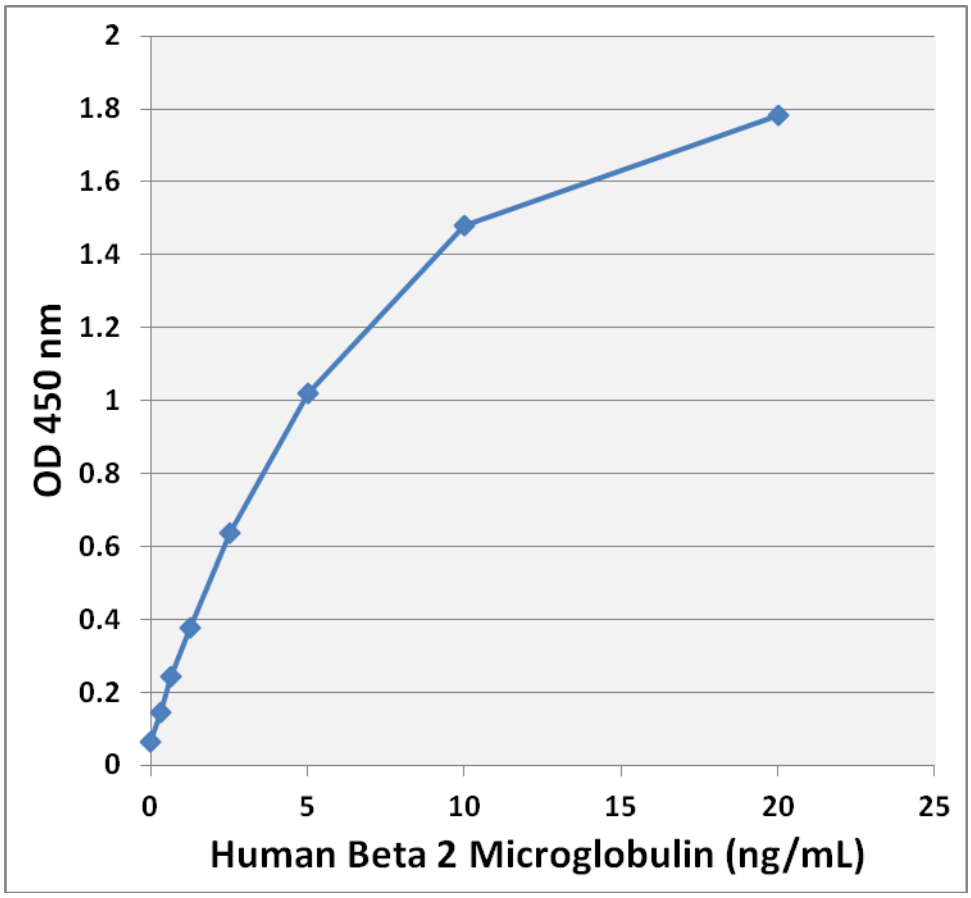

Figure 1: Human Beta 2 Microglobulin ELISA Standard Curve.

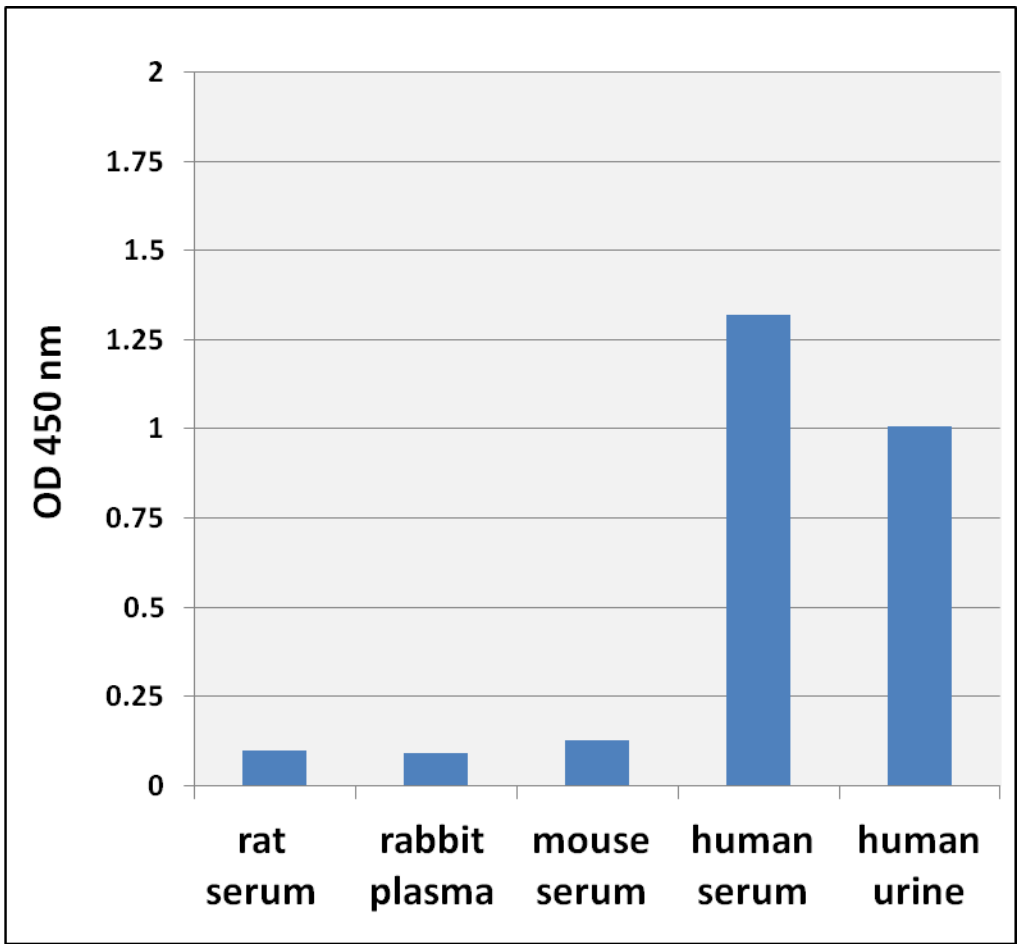

Figure 2: Detection of Beta 2 Microglobulin in plasma, serum, or urine. Each serum or plasma sample was diluted 200 fold and urine was diluted 16 fold according to the protocol above. Diluted samples were then tested using the Human Beta 2 Microglobulin ELISA Kit.

References

1. Güssow D, Rein R, Ginjaar I, Hochstenbach F, Seemann G, Kottman A, Ploegh HL (1987). *J. Immunol.* **139**: 3132–8.
2. Tysoe-Calnon VA, Grundy JE, and Perkins SJ. (1991) *Biochem. J.* **277**:359-369.
3. Otten GR, Bikoff E, Ribaudo RK, Kozlowski S, Margulies DH, Germain RN. (1992) *J. Immunol*. **148**:3723-32.
4. Neefjes JJ, Hämmerling GJ, Momburg F. (1993*) J. Exp Med*. **178**:1917-1980
5. Stephens AS, Stephens SR, Morrison NA (2011) *BMC Res Notes*. **4**:410-418.
6. Joshua DE1, Brown RD, Gibson J. (1994) *Leuk Lymphoma*. **15**:375-381

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED

WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2016-2017: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.