Datasheet for ABIN624946

beta-2 Microglobulin ELISA Kit

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Overview

Quantity:	96 tests
Target:	beta-2 Microglobulin (B2M)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	6-1000 pg/mL
Minimum Detection Limit:	6 pg/mL
Application:	ELISA

Product Details

Purpose:	Human Beta-2 Microglobulin ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with the rmbetaIGH3
Sensitivity:	< 6 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments • Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data

Product Details

Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard • Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	beta-2 Microglobulin (B2M)
Alternative Name:	Beta2M (B2M Products)
Background:	Beta-2-M (Beta2-microgiobulin) is found in the serum of normal individuals and in the urine in elevated amounts in patients with Wilson disease, cadmium poisoning, and other conditions leading to renal tubular dysfunction. It is produced by many cell types. Beta2 M plays an essential role both in governing MHC class I molecules stability and in promoting antigen binding and presenting the antigen to CD3/TCR complex of CD8 T cells. Beta-2-M and components of the major histocompatibility complex interact with hormone and other receptors for growth factors. Beta-2-M therefore acts as a growth factor and also modulates the binding of other growth factors to their receptors. The Human Beta2M ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Beta2M in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human Beta2M coated on a 96-well plate. Standards and samples are pipetted into the wells and Beta2M present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Beta2M antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Beta2M bound. The Stop

Target Details

	Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	567
UniProt:	P61769
Pathways:	TCR Signaling, Regulation of Leukocyte Mediated Immunity, Positive Regulation of Immune Effector Process

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples10,000-100,000 fold
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared biotin antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared Streptavidin solution to each well. 7. Incubate 45 min at RT. 8. Add 100 µL of TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL of Stop Solution to each well. 11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1 x Assay Diluent B (Item E) should be used for dilution of culture supernantants and urine. Suggested dilution for normal serum/plasma: 10,000-100,000 fold*. *Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator. 3. Assay Diluent B should be diluted 5-fold with deionized or distilled water. 4. Preparation of standard: Briefly spin the vial of Item C and then add 400 µL Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine, Assay Diluent B should be diluted 5-fold with deionized or distilled water) into Item C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 20 µL Beta2M standard from the vial of Item C, into a tube with 980 µL Assay Diluent A or 1x Assay Diluent B

	to prepare a 1,000 pg/mL stock standard solution. Pipette 400 µL Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL). 400 µL 400myl 400 µL 400 µL 400 µL 400 µL 20 µL standard + 980 µL 1,000 500 250 125 62.5 31.3 15.6 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 300-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 40 µL of HRP-Streptavidin concentrate into a tube with 12 ml 1x Assay Diluent B to prepare a final 300 fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day use). Mix well.
Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

Application Details

	9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points. Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent A Human Beta2 M concentration (pg/mL) O D =4 50 n m 0.1 1 10 10 100 1000 1000 Assay Diluent B Human Beta2 M concentration (pg/mL) O D =4 50 n m 0.1 1 10 10 100 1000 10000 Sensitivity: The minimum detectable dose of Beta2M is typically less than 6 pg/mL. Recovery: Recovery was determined by spiking various levels of human Beta2M into human serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 94.28 80-103 Plasma 95.78. 81-105 Cell culture media 104.6 93-118 Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 103 95 94 Range (%) 91-113 87-108 84-106 1:4 Average % of Expected 98 98 97 Range (%) 90-111 88-110 85-108 1:8 Average % of Expected 93 92 91 Range (%) 86-107 84-106 82-104 Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Publications

Product cited in:	Cassidy, Slyne, OKelly, Traynor, Conlon, Johnston, Slattery, Ryan, McMorrow: "Urinary biomarkers of chronic allograft nephropathy." in: **Proteomics. Clinical applications**, Vol. 9, Issue 5-6, pp. 574-85, (2015) (PubMed).

Publications

Ertugrul, Sahin, Dikilitas, Alpaslan, Bozoglan: "Evaluation of beta-2 microglobulin and alpha-2 macroglobulin levels in patients with different periodontal diseases." in: **Australian dental journal**, Vol. 58, Issue 2, pp. 170-5, (2013) (PubMed).

Gejyo, Arakawa: "Beta 2-microglobulin-associated amyloidoses." in: **Journal of internal medicine**, Vol. 232, Issue 6, pp. 531-2, (1993) (PubMed).

Gejyo, Yamada, Odani, Nakagawa, Arakawa, Kunitomo, Kataoka, Suzuki, Hirasawa, Shirahama: " A new form of amyloid protein associated with chronic hemodialysis was identified as beta 2-microglobulin." in: **Biochemical and biophysical research communications**, Vol. 129, Issue 3, pp. 701-6, (1985) (PubMed).

Arce-Gomez, Jones, Barnstable, Solomon, Bodmer: "The genetic control of HLA-A and B antigens in somatic cell hybrids: requirement for beta2 microglobulin." in: **Tissue antigens**, Vol. 11, Issue 2, pp. 96-112, (1978) (PubMed).

Images

ELISA

Image 1.