



AssayMax Human β_2 -Microglobulin ELISA Kit

Catalog # EM5001-1

Introduction

β_2 -Microglobulin (β_2 M) is a small serum protein that constitutes the light chain of the major histocompatibility class I human leukocyte antigen (HLA class I), an integral membrane protein involved in the immune response. The protein is 99 amino acid residues in length and has molecular mass of 12 kDa (1 - 4). β_2 M is released from the cell surface of HLA class I into the serum and carried to the kidneys for degradation and secretion (5). In chronic renal failure, β_2 M accumulates as insoluble amyloid aggregates and causes arthralgias, destructive osteoarthropathies, carpal tunnel syndrome and dialysis-related amyloidosis (6 - 8). Elevated serum β_2 M levels are associated with poor prognosis in multiple myeloma and lymphoma (9 - 12).

Principal of the Assay

The AssayMax Human β_2 -Microglobulin ELISA kit is designed for detection of human β_2 -Microglobulin in plasma, serum, urine and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures β_2 -Microglobulin in 4 hours. A polyclonal antibody specific for β_2 -Microglobulin has been pre-coated onto a microplate. β_2 -Microglobulin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for β_2 -Microglobulin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **β_2 -Microglobulin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human β_2 -Microglobulin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **β_2 -Microglobulin Standard:** human β_2 -Microglobulin in a buffered protein base (200 ng, lyophilized).

- **Biotinylated β_2 -Microglobulin Antibody (100x):** A 100-fold biotinylated polyclonal antibody against β_2 -Microglobulin (80 μ l).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 μ l).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2 - 8⁰C. Store reconstituted reagents at -20⁰C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 μ l, 20-200 μ l, 200-1000 μ l and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:2000 into MIX Diluent. Add 5 μ l of sample to 495 μ l of MIX Diluent (1:100) to make Solution A; then add 25 μ l of Solution A to 475 μ l of MIX Diluent (1:20) to make a final working solution (1:2000). The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:2000 into MIX Diluent. Add 5 μ l of sample to 495 μ l of MIX Diluent (1:100) to make Solution A; then add 25 μ l of Solution A to 475 μ l of MIX Diluent (1:20) to make a final working solution (1:2000). The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20⁰C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:100 into MIX Diluent. Store samples at -20⁰C or below for up to 1 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2 - 8°C.
- **β_2 -Microglobulin Standard:** Reconstitute the 200 ng of human β_2 -Microglobulin Standard with 1 ml of MIX Diluent to generate a standard solution of 200 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Standard solution (200 ng/ml) 1:4 with MIX Diluent to produce 50, 12.5, 3.13 and 0.78 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[b2M] (ng/ml)
P1	1 part Standard (200 ng/ml)	200.00
P2	1 part P1 + 3 part MIX Diluent	50.00
P3	1 part P2 + 3 part MIX Diluent	12.50
P4	1 part P3 + 3 part MIX Diluent	3.13
P5	1 part P4 + 3 part MIX Diluent	0.78
P6	MIX Diluent	0.00

- **Biotinylated β_2 -Microglobulin Antibody (100x):** Spin down the biotinylated antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 μ l of standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 μ l of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove liquid at each step.
- Add 50 μ l of Biotinylated β_2 -Microglobulin Antibody to each well and incubate for one hour.
- Wash five times with 200 μ l of Wash Buffer.
- Add 50 μ l of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 μ l of Wash Buffer.
- Add 50 μ l of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μ l of Stop Solution to each well. The color will change from blue to yellow.

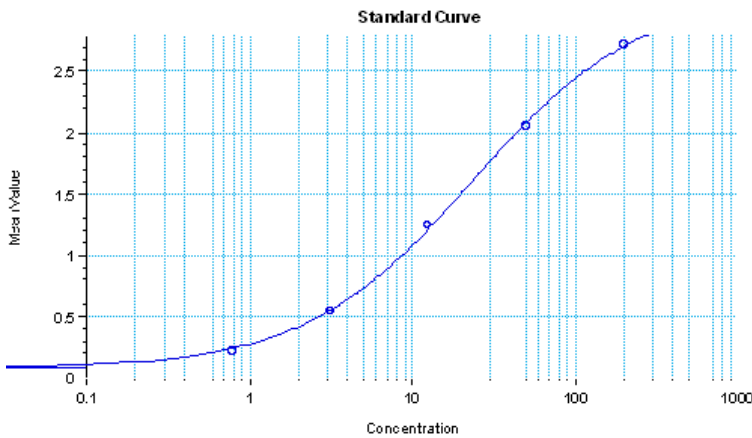
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of β_2 -Microglobulin is typically 0.7 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9 % and 7.2% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:1000	95%	97%
1:2000	98%	101%
1:4000	105%	109%

Sample Dilution	Urine
1:50	100%
1:100	98%
1:200	101%

Recovery

Standard Added Value	1 – 50 ng/ml
Recovery %	85-110 %
Average Recovery %	97.5 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	< 0.2
Monkey	> 40 (Suggest 1:500 dilution for plasma/serum samples)
Mouse	< 1
Rat	< 0.5
Swine	None
Bovine	None
Rabbit	None

Reference Value

- The normal serum levels of b2M is less than 2.7 ug/ml, and urine levels of b2M is less than 200 ng/ml.

References

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