Quantikine® IVD® ELISA

Human β_2M Immunoassay

Catalog Number DBM200

Enzyme immunoassay (EIA) for the quantitative determination of beta 2-Microglobulin (β_2M) concentration in human serum and urine as an aid in the diagnosis of active rheumatoid arthritis and kidney diseases.

This package insert must be read in its entirety before using this product.
For *In Vitro* Diagnostic Use.

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NAME & INTENDED USE

Quantikine® IVD® Human β_2M ELISA
R&D Systems Inc. Catalog Number DBM200

Enzyme immunoassay (EIA) for the quantitative determination of β_2-Microglobulin (β_2M) concentration in human serum and urine as an aid in the diagnosis of active rheumatoid arthritis and kidney diseases.

INTRODUCTION

β_2M is a 12 kDa, 99 amino acid polypeptide that forms the light chain of Class I MHC molecules (1, 2). Possessing an Ig-like domain, β_2M noncovalently associates with both 44 kDa classical (HLA-A, -B & -C) and 40 kDa non-classical (HLA-E, -F & -G) Class I MHC heavy chains as well as 43-49 kDa Class I non-MHC heavy chains (CD-1) (2-6). β_2M is normally found on nearly all nucleated cells, with neurons a notable exception (7, 8).

Circulating β_2M is generated during normal HLA turnover (9). It has been measured in a variety of body fluids, including serum, plasma, saliva, CSF, and urine (10, 11). β_2M freely passes through the glomerular membrane, but it is 99% actively reabsorbed and degraded in the proximal tubule cells. Clinically, serum β_2M has been noted to be increased in rheumatoid arthritis, systemic lupus erythematosus, viral infections and conditions with decreased glomerular filtration (12, 13).

PRINCIPLE OF THE ASSAY

The Quantikine IVD Human β_2M EIA is based on the competitive binding enzyme immunoassay technique. β_2M in the patient sample or standard competes with a fixed amount of enzyme-labeled β_2M for sites on a mouse monoclonal antibody. During the reaction the monoclonal antibody becomes bound to anti-mouse antibodies coated onto the microplate.

Excess conjugate and unbound sample or standard are washed from the plate, and the bound enzyme activity is determined by the addition of substrate to the wells. The reaction is halted with the addition of Stop Solution and the absorbance of the contents of the wells is read at 450 nm. The absorbance is inversely proportional to the concentration of β_2M in the sample.

LIMITATIONS OF THE PROCEDURE

- FOR *IN VITRO* DIAGNOSTIC USE.
- The results of this assay should be used in conjunction with information available from clinical evaluations and other diagnostic procedures.
- The kit should not be used beyond the expiration date on the kit label.
- If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay.
- Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time or temperature, or kit age can cause variation in binding.

TECHNICAL HINTS

- Allow samples and all reagents to reach room temperature prior to performing the assay. This usually takes about an hour. It is essential that the pouch containing the microplate is allowed to warm to ambient temperature and is free of condensation prior to opening to avoid moisture entering the wells. The desiccant pack should remain within the pouch at all times, and the pouch should be sealed to maintain a dry environment.
- Gently mix samples and all reagents before use. Avoid vigorous agitation and foaming.
- Avoid handling the tops of the wells. Keep the wells covered with a plate sealer except when adding reagents, washing or reading.
- To avoid microbial or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips or pipettes.
- For partial use of the kit, transfer an appropriate volume of each reagent to a clean (preferably disposable) vessel, free from detergent residues, etc., for subsequent dispensing into the microplate wells. This reduces the possibility of reagent contamination by repeated sampling from the original bottles.
- Avoid contact of Substrate Solution with oxidizing agents or metals.
- Sodium azide will inactivate the β_2M-HRP Conjugate.
- It is recommended that samples, Standards and Control Sera are assayed in duplicate.
- A standard curve must be run with each assay.
- Additions at each step of the protocol should be uninterrupted.
- Pipette slowly and carefully to avoid reagent splashing.
- Do not mix or substitute reagents with those from other lots or sources.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.
Expiration dates are stated on the kit box and vial labels.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
β_2M Microplate	895110	96 well polystyrene microplate (12 strips of 8 wells) coated with goat anti-mouse IgG polyclonal antibody.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 4 weeks at 2-8 °C.*
β_2M Standard Set	895131 & 895111-895115	6 vials containing human β_2M in 0.4 mL of buffered animal serum with preservatives.	
β_2M Control Sera	895123-895124	2 vials containing human β_2M in 0.4 mL of buffered animal serum with preservatives.	
β_2M Conjugate Concentrate	895116	0.3 mL of a concentrated solution of β_2M-HRP conjugate in a buffered protein solution with preservatives.	
Conjugate Diluent	895108	11 mL of buffered protein solution with yellow dye and preservatives.	
Sample Diluent	895109	5 mL of buffered animal serum with preservatives.	May be stored for up to 4 weeks at 2-8 °C.*
Antibody Solution	895133	11 mL of monoclonal antibody to β_2M in a buffered protein solution with blue dye and preservatives.	
Wash Buffer Concentrate	895125	20 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Substrate	895136	11 mL of stabilized substrate solution (tetramethylbenzidine).	
Stop Solution	895132	11 mL of 1 N HCl. Ready to use. **Caution:** *Caustic material. Wear eye, hand, face, and clothing protection.*	
Plate Sealers	N/A	8 adhesive strips.	
Data Card	N/A	Provides ranges of controls	

* Provided this is within the expiration date shown on the kit box and the vial labels.

OTHER SUPPLIES REQUIRED

- 1 N NaOH (for pH adjustment of urine samples).
- Precision pipettes to deliver 20-1000 µL.
- 500 mL graduated cylinder or volumetric flask.
- Washing device suitable for microplates.
- Microplate reader capable of measuring absorbance at 450 nm with dual wavelength correction set at 620 nm.
- Distilled or deionized water.
- A computer capable of 4-parameter logistic curve fitting for data reduction.

PRECAUTIONS

All blood and urine derivatives should be considered potentially infectious.

This kit contains β_2M obtained from human urine. The urine pool has been tested by an FDA licensed method for HIV 1/2 antibodies and Hepatitis B surface antigen and found to be non-reactive. Since no known test method can rule out the possibility of infection, this material should be handled as if it is capable of transmitting disease.

Do not pipette by mouth.

Do not smoke, eat, or drink in areas where specimens or reagents are being handled.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

The Stop Solution provided with this kit is an acid solution.

Wear protective gloves, clothing, eye, and face protection when handling reagents and specimens. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

Serum - A minimum of 0.25 mL of blood per assay should be collected using standard aseptic phlebotomy techniques. 40 µL (2 replicates) of serum is needed for each assay. If not assayed immediately samples can be stored for up to 4 weeks at ≤ -20 °C. Do not use lipemic, grossly hemolyzed or turbid specimens. **Avoid repeated freeze-thaw cycles.**

Urine - Specimens should be processed within 1 hour of collection. β_2M will degrade in acidic conditions (below pH 5.5); therefore, the pH of the urine must be checked and adjusted to between pH 6-8 with the addition of 1 N NaOH. Neutralized urine may be stored for up to 4 weeks at ≤ -20 °C, if testing is not carried out on day of collection. Avoid repeated freeze-thaw cycles.

Note: *Sodium azide must not be used to preserve samples as it has an inhibitory effect on peroxidase.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Conjugate Concentrate - Tap the vial of Conjugate Concentrate to dislodge any excess material from the cap. Transfer 250 µL of Conjugate Concentrate into the bottle of Conjugate Diluent. Mix contents by gentle inversion and swirling. **Vigorous agitation and foaming should be avoided.**

Wash Buffer Concentrate - If crystals have formed in the concentrate, warm and mix gently until the crystals have completely dissolved. Pour the contents of the Wash Buffer Concentrate vial into a clean graduated measuring cylinder and bring the final volume to 500 mL with distilled or deionized water.

All other reagents are ready for use.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents as directed in the previous sections.
2. Open the resealable ziplock bag containing the microplate by tearing at the notches. Remove any excess microplate strips from the plate frame and store them in the resealable bag with the desiccant pack. Reseal the bag.
3. Add 20 µL of each Standard, Control, or sample to the appropriate wells.
4. Add 100 µL of Conjugate Solution (yellow) to each well of the plate.
5. Add 100 µL of Antibody Solution (blue) to each well of the plate.
6. Cover the plate with a plate sealer provided and incubate at room temperature for 1 hour.
7. Aspirate or decant contents from each well and wash by adding 400 µL of Wash Buffer per well. Repeat the process to give six washes in total. After the last wash, remove any remaining Wash Buffer by inverting the plate and tapping it firmly on clean paper towelling.
8. Immediately after decanting, add 100 µL of Substrate to each well.
9. Cover the plate with a plate sealer provided and incubate at room temperature for 15 minutes.
10. Add 100 µL of Stop Solution to each well. The Stop Solution should be added to the wells in the same order as the Substrate.
11. Determine the optical density of each well within 30 minutes of addition of Stop Solution, using a microplate reader or photometer set at 450 nm with a correction wavelength of 620 nm. The plate reader or photometer should be blanked according to the manufacturer's instructions. If the wavelength correction capability is not available, subtract readings at 620 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the polystyrene microplate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Calculate the mean absorbance values for each set of duplicate standards.

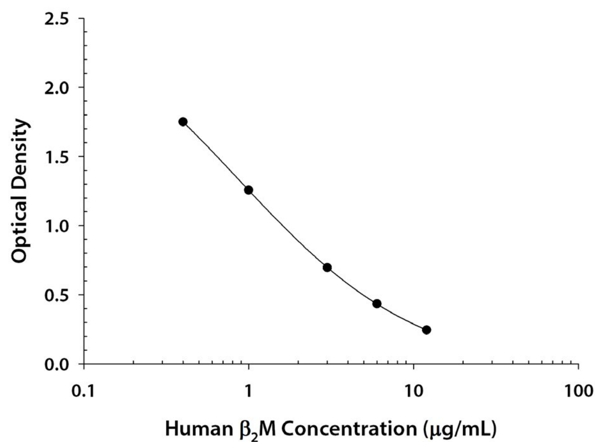
Create a standard curve by reducing the data using software capable of generating a four parameter logistic (4-PL) curve-fit.

Determine the concentration of each unknown sample by calculating the concentration of β_2M corresponding to the mean absorbance from the standard curve. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

It is recommended that the user run the Controls in each assay. If the values obtained are not within the expected ranges, the assay results may be invalid.

TYPICAL DATA

This graph and the optical density (O.D.) data are shown as an example. A standard curve must be generated each time the assay is run.



Standard (µg/mL)	O.D.	Average
0	2.207	2.206
0	2.204	
0.4	1.735	1.750
0.4	1.765	
1.0	1.240	1.256
1.0	1.273	
3.0	0.722	0.696
3.0	0.671	
6.0	0.445	0.434
6.0	0.422	
12.0	0.251	0.245
12.0	0.239	

QUALITY CONTROL

Each testing laboratory should establish a quality control program to monitor the performance of the Quantikine IVD Human β_2M Immunoassay. As part of this quality control program, controls of known β_2M concentration should be run in each assay (supplied in kit or purchased commercially). Two control sera are supplied in the kit. If the values obtained are not within their established ranges, the assay results may be invalid.

TROUBLESHOOTING GUIDE

On the occasion of an assay failure, check the expiration dates of the individual reagents and ensure that all the reagents have been stored as indicated on the product labeling. If assay performance is questionable or a problem occurs when running the assay, you may be able to isolate the problem by referring to the following table.

PROBLEM	POSSIBLE SOURCE	TEST OR ACTION
Poor Precision	Incomplete washing of wells	Ensure that the wash station is working properly
	Inadequate aspiration of the wells	Wells should appear dry after aspiration
	Unequal volumes added to the wells	Ensure that the pipette is working properly
	Reagent splashing during addition or plate handling	Pipette slowly and carefully. Take care not to bump the filled plate
Poor Curve Fit	Pipetting error	Consider editing data according to individual laboratory procedures
Inadequate Color Development	Inadequate aspiration of the wells	Wells should appear dry after aspiration
	Incorrect volumes added to the wells	Ensure that the pipette is working properly
	Incorrect incubation times or temperatures	Adhere to recommended incubation periods and temperatures
	Conjugate or Substrate failure	Mix equal volumes (*i.e.* 100 µL each) of Substrate and Conjugate. Color should develop immediately

DILUTION OF SAMPLES WITH HIGH β_2M CONCENTRATIONS

If a serum or urine sample is suspected of being above 12 µg/mL, dilute it with the Sample Diluent.

To determine the β_2M concentration of the serum or urine sample, multiply the result obtained by the dilution factor employed.

PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

	Serum Intra-Assay Precision			Urine Intra-Assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	10	10	10
Mean (µg/mL)	0.9	1.4	8.4	1.2	3.3	8.6
CV (%)	8.1	6.3	5.3	7.4	5.3	4.4

Inter-assay Precision (Precision between assays)

Samples of known concentration were tested in duplicate in forty separate assays on 20 days using 3 kit lots to assess inter-assay precision.

	Serum Inter-Assay Precision						Urine Inter-Assay Precision		
Sample	1	2	3	4	5	6	1	2	3
n	40	40	40	40	40	40	40	40	40
Mean (µg/mL)	0.9	1.3	8.9	1.1	3.2	8.0	1.1	3.4	8.7
CV (%)	9.2	6.8	4.8	8.9	6.9	6.0	9.2	5.6	4.9

RECOVERY

The recovery of β_2M spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Normal human serum	98	87-113%
Urine	105	87-113%

LINEARITY

Samples were assayed at serial two-fold dilutions. The data below are typical of the assay.

		Serum (n=5)	Urine (n=5)
1:2	Average % of Expected	102	100
	Range (%)	98-106	91-108
1:4	Average % of Expected	100	95
	Range (%)	93-103	87-101
1:8	Average % of Expected	95	96
	Range (%)	85-103	91-99
1:16	Average % of Expected	96	93
	Range (%)	84-109	82-108

SENSITIVITY

The sensitivity (minimum detectable dose) of the Quantikine IVD Human β_2M EIA was determined to be less than 0.2 μg/mL. This was determined by subtracting two standard deviations from the mean optical density of 20 replicates of the zero standard and calculating the corresponding concentration from a linear regression plot between the mean O.D. reading of the zero standard and the first non-zero standard.

CALIBRATION

The kit was calibrated against the 1st International Standard for β_2M (1985).

EXPECTED VALUES

Serum - Samples from apparently healthy normal donors were tested for β_2M concentration. The ranges differed between male and female donors as follows:

Sample Type	Mean (μg/mL)	Range (μg/mL) (2.5-97.5 percentile)
Male serum (n=73)	1.7	1.1-2.6
Female serum (n=84)	1.6	1.1-2.3

Urine - A panel of 26 urine samples from apparently normal donors (16 males and 10 females) was tested for β_2M concentration. All values were < 0.2 μg/mL.

Note: *These values are given for guidance purposes only. Laboratories should determine their own reference ranges.*

CROSS-REACTIVITY

The Quantikine IVD Human β_2M EIA exhibits no significant cross-reactivity as recorded below:

Human IgG up to 100 mg/mL

Human IgA up to 10 mg/mL

Human Albumin up to 200 mg/mL

Retinol binding protein has been found to cross-react 0.46% at 50% displacement.

METHOD COMPARISON

The Quantikine IVD Human β_2M was compared to a commercially available β_2M RIA by measuring β_2M in 103 sera and 76 urine (28 positive and 48 undetectable) samples. Regression analyses gave the relationships:

In Serum:
Quantikine IVD Human β_2M = 1.16 x competitor assay - 0.014
The correlation coefficient was 0.970

In Urine:
Quantikine IVD Human β_2M = 1.09 x competitor assay - 0.038
The correlation coefficient was 0.997

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