

# Parameter™

## Human $\beta$ 2-Microglobulin Assay

Catalog Number KGE019

For the quantitative determination of human beta 2-Microglobulin ( $\beta$ 2M) in serum, plasma, and urine.

This package insert must be read in its entirety before using this product.  
For research use only. Not for use in diagnostic procedures.

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## INTRODUCTION

Beta 2-Microglobulin ( $\beta$ 2M) is a widely expressed 12 kDa secreted polypeptide that serves as the light chain of Class I MHC molecules (1).  $\beta$ 2M noncovalently associates with classical (HLA-A, -B, -C) and non-classical (HLA-E, -F, -G) Class I MHC heavy chains and with Class I non-MHC heavy chains (CD1) (2-4).  $\beta$ 2M is required for the proper folding, binding of antigenic peptide, and surface expression of MHC-I molecules (5-10) as well as the development of peripheral CD4-CD8+ T cells (7, 8). It also promotes the cell surface expression of FcRN and the MHC-I like molecules MILL1 and MICB/MILL2 (10, 11). Mature human  $\beta$ 2M shares 70% and 75% amino acid sequence identity with mouse and rat  $\beta$ 2M, respectively (12).

$\beta$ 2M can dissociate from the MHC and circulate in complex with alpha 2-Macroglobulin (13). It is present in a variety of body fluids, and it is elevated in rheumatoid arthritis, systemic lupus erythematosus, viral infections, cancer, aging, and conditions with decreased glomerular filtration (14-17).  $\beta$ 2M freely passes through the glomerular membrane, but it is 99% actively reabsorbed and degraded in the proximal tubule cells (18). It circulates at elevated levels in chronic kidney dialysis patients, leading to systemic amyloidosis (19). In these patients, the N-terminal 6 aa of  $\beta$ 2M can be trimmed off, inducing it to form multimers and become deposited in amyloid fibrils (20-23). Fibril formation is enhanced by  $\beta$ 2M interaction with heparin and type I collagen fibers (24, 25).

$\beta$ 2M regulates cellular iron metabolism through binding to the MHC-I like HFE protein, although this is prevented in hereditary hemochromatosis (HH) patients who harbor mutations in HFE (26-29). It also binds to IGF-I and enhances IGF-I activity (30).  $\beta$ 2M promotes mesenchymal stem cell proliferation, epithelial mesenchymal cell transition, and tumor progression (26, 31, 32). Its elevation during aging impairs cognition, memory formation, and hippocampal regeneration (16).

The Parameter  $\beta$ 2-Microglobulin Immunoassay is a 1.5 hour forward sequential competitive enzyme immunoassay designed to measure  $\beta$ 2M in serum, plasma, and urine.

## PRINCIPLE OF THE ASSAY

This assay is based on the forward sequential competitive binding technique in which  $\beta$ 2M present in a sample competes with horseradish peroxidase (HRP)-labeled  $\beta$ 2M for a limited number of binding sites on a mouse monoclonal antibody.  $\beta$ 2M in the sample is allowed to bind to the antibody in the first incubation. During the second incubation, HRP-labeled  $\beta$ 2M binds to the remaining antibody sites. Following a wash to remove unbound materials, a substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped, and the absorbance is read at 450 nm. The intensity of the color is inversely proportional to the concentration of  $\beta$ 2M in the sample.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, incubation time or temperature, and kit age can cause assay variability.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Pre-rinse the pipette tips when pipetting.
- Pipette standards and samples to the bottom of the wells. Add all other reagents to the side of the wells to avoid contamination.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution. Gently tap the side of the plate to ensure thorough mixing.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Goat Anti-mouse IgG Microplate	892575	96 well polystyrene microplate (12 strips of 8 wells) coated with a goat anti-mouse polyclonal antibody.	Return unused wells to the storage bag. May be stored at 2-8 °C for up to 1 month.*
Human $\beta$ 2M Standard	898445	2 vials of human $\beta$ 2M in a buffer with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Use and discard. Use a fresh standard for each assay.
Human $\beta$ 2M Conjugate Concentrate, 45X	898462	0.3 mL/vial of a concentrated solution of $\beta$ 2M conjugated to horseradish peroxidase in a buffered protein base with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Conjugate Diluent 32	896330	11 mL of buffered protein solution with yellow dye and preservatives.	
Human $\beta$ 2M Primary Antibody Solution	898463	11 mL of a mouse monoclonal antibody to $\beta$ 2M in a buffered protein base with blue dye and preservatives.	
Calibrator Diluent RD5-73	896331	11 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	Adhesive strips.	

\* Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.
- Human  $\beta$ 2M Controls (optional; R&D Systems, Catalog # QC224).

## PRECAUTIONS

$\beta$ 2M is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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

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

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

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

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Note:** *Citrate plasma has not been validated for use in this assay.*

**Urine** - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

## SAMPLE PREPARATION

Urine samples require a 2-fold dilution due to a matrix effect. A suggested 2-fold dilution is 50  $\mu$ L of sample + 50  $\mu$ L of Calibrator Diluent RD5-73.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Note:**  $\beta 2M$  is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

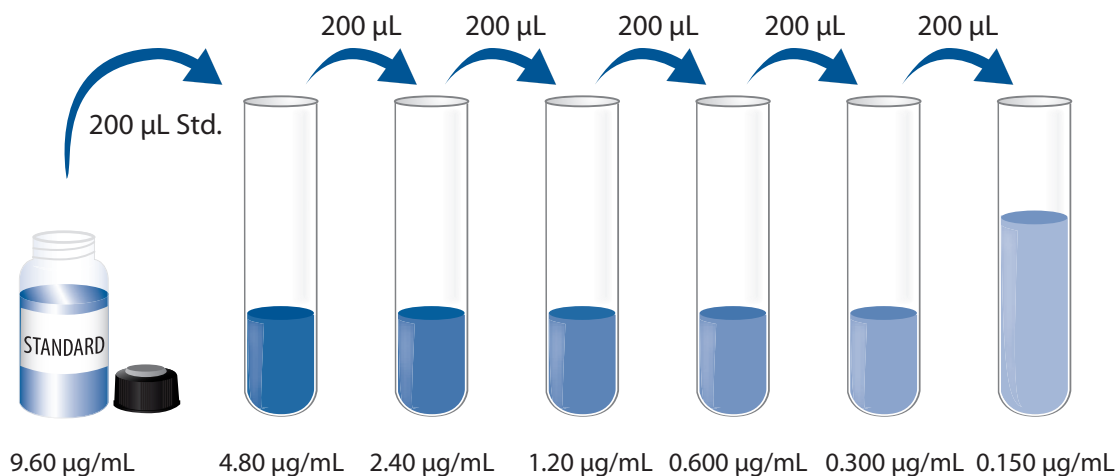
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200  $\mu$ L of the resultant mixture is required per well.

**Human  $\beta 2M$  Conjugate** - Tap the vial of Human  $\beta 2M$  Conjugate Concentrate, 45X to dislodge any excess material from the cap. Transfer 250  $\mu$ L of Human  $\beta 2M$  Conjugate Concentrate, 45X into Conjugate Diluent 32. Mix with gentle inversion or swirling. **Vigorous agitation and foaming should be avoided.**

**Human  $\beta 2M$  Standard - Refer to the vial label for reconstitution volume.** Reconstitute the Human  $\beta 2M$  Standard with deionized or distilled water. This reconstitution produces a 1X ready-to-use stock solution of 9.60  $\mu$ g/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 30 minutes with gentle agitation prior to making dilutions.

Pipette 200  $\mu$ L of Calibrator Diluent RD5-73 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The Human  $\beta 2M$  Standard (9.60  $\mu$ g/mL) serves as the high standard. Calibrator Diluent RD5-73 serves as the zero standard (0  $\mu$ g/mL).



## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.**

$\beta$ 2M is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 20  $\mu$ L of standard, control, or sample\* per well
4. Add 100  $\mu$ L of Human  $\beta$ 2M Conjugate prepared as described in the Reagent Preparation section to each well.
5. Add 100  $\mu$ L of Human  $\beta$ 2M Primary Antibody Solution to each well. Cover with an adhesive strip. Incubate for 1 hour at room temperature.
6. Aspirate each well and wash, repeating the process five times for a total of six washes. Wash by filling each well with Wash Buffer (400  $\mu$ L) using a squirt bottle, manifold dispenser, 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.
7. Add 200  $\mu$ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
8. Add 50  $\mu$ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

\*Sample may require dilution. See Sample Preparation section.



## CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a logarithmic x-axis and draw the best fit curve through the points on the graph. Do not include the  $B_0$  in the standard curve.

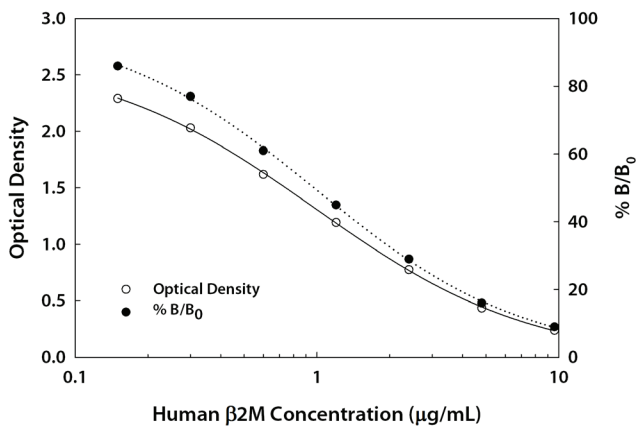
If desired, % B/ $B_0$  can be calculated by dividing the average optical density (O.D.) for each standard or sample by the average  $B_0$  O.D. and multiplying by 100.

Calculate the concentration of human  $\beta 2M$  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.

## TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



( $\mu\text{g/mL}$ )	O.D.	Average	% B/ $B_0$
0 ( $B_0$ )	2.562 2.744	2.653	—
0.150	2.267 2.316	2.292	86
0.300	1.981 2.081	2.031	77
0.600	1.617 1.623	1.620	61
1.20	1.125 1.263	1.194	45
2.40	0.728 0.821	0.775	29
4.80	0.423 0.446	0.435	16
9.60	0.227 0.248	0.238	9

## PRECISION

### Intra-assay Precision (Precision within an assay)

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

### Inter-assay Precision (Precision between assays)

Three controls of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (µg/mL)	1.14	3.19	6.41	0.953	3.10	6.17
Standard deviation	0.086	0.189	0.383	0.175	0.293	0.496
CV (%)	7.5	5.9	6.0	18.4	9.5	8.0

## RECOVERY

The recovery of human  $\beta$ 2M spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Serum (n=4)	101	85-122%
EDTA plasma (n=4)	99	79-114%
Heparin plasma (n=4)	106	91-124%
Urine* (n=4)	90	76-105%

\*Samples were diluted prior to assay as directed in the Sample Preparation section.

## SENSITIVITY

Thirty-six assays were evaluated and the minimum detectable dose (MDD) of human  $\beta$ 2M ranged from 0.016-0.132 µg/mL. The mean MDD was 0.069 µg/mL.

The MDD was determined by subtracting two standard deviations from the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## CALIBRATION

This kit was calibrated against the 1st International Standard for  $\beta$ 2M (1985).

## LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human  $\beta$ 2M were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine* (n=4)
1:2	Average % of Expected	93	91	95	109
	Range (%)	87-97	88-97	82-101	104-114
1:4	Average % of Expected	88	93	97	110
	Range (%)	84-96	80-99	78-117	105-114
1:8	Average % of Expected	90	97	101	94
	Range (%)	85-102	80-108	76-112	89-100

\*Samples were diluted prior to assay as directed in the Sample Preparation section.

## SAMPLE VALUES

**Serum/Plasma** - Samples from apparently healthy volunteers were evaluated for the presence of human  $\beta$ 2M in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean ( $\mu$ g/mL)	Range ( $\mu$ g/mL)	Standard Deviation ( $\mu$ g/mL)
Serum (n=30)	2.53	1.68-4.13	0.668
EDTA plasma (n=30)	2.31	1.29-4.10	0.701
Heparin plasma (n=30)	2.27	1.26-3.99	0.673

**Urine** - Ten samples from apparently healthy volunteers were evaluated for the presence of human  $\beta$ 2M in this assay. No detectable levels were observed. No medical histories were available for the donors used in this study.

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## SPECIFICITY

The factors listed below were prepared in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors in a mid-range human  $\beta$ 2M control were assayed for interference. No significant cross-reactivity or interference was observed

### Recombinant human:\*

AFP  
Albumin  
RBP-4

### Recombinant mouse:\*\*

$\beta$ 2-Microglobulin

### Natural proteins:\*

human AFP  
human IgA  
human IgG

\*Prepared at 100 mg/mL.

\*\*Prepared at 20 mg/mL.

## REFERENCES

1. Li, L. *et al.* (2016) *Chin. Med. J.* **129**:448.
2. Ulbrecht, M. *et al.* (1992) *J. Immunol.* **149**:2945.
3. Houlihan, J.-M. *et al.* (1992) *J. Immunol.* **149**:668.
4. Paduraru, C. *et al.* (2006) *J. Biol. Chem.* **281**:40369.
5. Vitiello, A. *et al.* (1990) *Science* **250**:1423.
6. Williams, D.B. *et al.* (1989) *J. Immunol.* **142**:2796.
7. Koller, B.H. *et al.* (1990) *Science* **248**:1227.
8. Zijlstra, M. *et al.* (1990) *Nature* **344**:742.
9. Robinson, P.J. *et al.* (1998) *J. Immunol.* **160**:3217.
10. Praetor, A. and W. Hunziker (2002) *J. Cell Sci.* **115**:2389.
11. Kajikawa, M. *et al.* (2006) *J. Immunol.* **177**:3108.
12. Suggs, S.V. *et al.* (1981) *Proc. Natl. Acad. Sci. USA* **78**:6613.
13. Motomiya, Y. *et al.* (2003) *Kidney Int.* **64**:2244.
14. McCarthy, J.T. *et al.* (1994) *J. Lab. Clin. Med.* **123**:495.
15. Svatonova, J. *et al.* (2014) *Dis. Markers* **2014**:495402.
16. Smith, L.K. *et al.* (2015) *Nat. Med.* **21**:932.
17. Jeffery, G.M. *et al.* (1990) *Pathology* **22**:20.
18. Hall, P.W. *et al.* (1980) *Vox Sang* **38**:343.
19. Stoppini, M. and V. Bellotti (2015) *J. Biol. Chem.* **290**:9951.
20. Esposito, G. *et al.* (2000) *Prot. Sci.* **9**:831.
21. Gorevic, P.D. *et al.* (1986) *Proc. Natl. Acad. Sci. USA* **83**:7908.
22. Linke, R.P. *et al.* (1989) *Kidney Int.* **36**:675.
23. Gorevic, P.D. *et al.* (1985) *J. Clin. Invest.* **76**:2425.
24. Relini, A. *et al.* (2008) *J. Biol. Chem.* **283**:4912.
25. Relini, A. *et al.* (2006) *J. Biol. Chem.* **281**:16521.
26. Jossen, S. *et al.* (2011) *Cancer Res.* **71**:2600.
27. Enns, C.A. (2001) *Traffic* **2**:167.
28. Waheed, A. *et al.* (1997) *Proc. Natl. Acad. Sci. USA* **94**:12384.
29. Feder, J.N. *et al.* (1997) *J. Biol. Chem.* **272**:14025.
30. Centrella, M. *et al.* (1989) *J. Biol. Chem.* **264**:18268.
31. Zhu, Y. *et al.* (2009) *Biotechnol. Lett.* **31**:1361.
32. Nomura, T. *et al.* (2014) *Anti-Cancer Agents Med. Chem.* **14**:343.

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