BOSTER

antibody and ELISA experts

# Human Beta-2 Microglobulin OneStep ELISA Kit

**Catalog number: EK7023**

For detection of multiple analytes using one single assay.

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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# Human Beta-2 Microglobulin OneStep ELISA Kit

**Catalog Number:** EK7023

**Size:** 96 wells/kit

**Sample Type:** Serum and Plasma

**Sensitivity:** 0.625 μg/ml

**Assay Range:** 0.625-10 μg/ml

**Storage:** Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

## Introduction

The Bosterbio OneStep ELISA kit is a solid phase sandwich ELISA kit. Instead of incubating with standards, samples and controls and with enzyme conjugated detection antibody separately, the OneStep ELISA kit allows the user to add standards, samples and controls to wells without incubation, and an enzyme conjugate reagent is added into each well immediately. Then incubate standards, samples, controls and enzyme conjugate reagent together. After the excess enzyme conjugate is washed out, the substrate is added into each well. The enzyme catalyzes the substrate yielding a blue color (Amax = 370nm and 652nm) that changes to yellow (Amax = 450nm) upon addition of a sulfuric or phosphoric acid stop solution. The intensity of color developed is directly proportional to the concentration of target protein in the samples. A standard curve is generated relating color intensity to the concentration of target protein.

## Kit Components

| Description | Quantity |
|---|---|
| Microwells coated Murine monoclonal anti-B2 MG antibody | 12x8x1 |
| B2MG Reference Standards: 0, 0.625, 1.25, 2.5, 5, and 10 | 1 ml |
| Sample Diluent, 100 ml. | 100 ml |
| Enzyme Conjugate Reagent, 22 ml | 22 ml |
| TMB Reagent (One-Step), 11 ml | 11 ml |
| Stop Solution (1N HCl), 11 ml. | 11 ml |
| Wash concentrate 20X: 1 bottle | 25 ml |

### Standard Concentrations and example data

| | B2MG ( g/ml) | Absorbance (450 nm) |
|---|---|---|
| Std 1 | 0 | 0.052 |
| Std 2 | 0.625 | 0.377 |
| Std 3 | 1.25 | 0.745 |
| Std 4 | 2.5 | 1.414 |
| Std 5 | 5 | 2.085 |

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| | | |
|---|---|---|
| Std 6 | 10 | 2.942 |

## Materials Required, but Not Provided

1. Distilled or deionized water

2. Precision pipettes

3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.

4. ELISA reader capable of reading absorbance at 450nm

5. Absorbance paper or paper towel

6. Graph paper

## WARNINGS AND PRECAUTIONS

1. Potential for Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory use.
3. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different kit lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care, since it is corrosive.
15. Bring all reagents to room temperature.
16. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
17. Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

## SPECIMEN COLLECTION AND HANDLING

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipidic or turbid samples.
2. Typicaly, specimens should be capped and may be stored for up to 48 hour at 2-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C for up to 6 months prior to assay. Thawed samples should be inverted several times to mix prior to testing.
3. Collect urine samples and store at 2-8°C for up to 5 days or at -20°C for longer periods. Urine samples are calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

## REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature

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(20-25°C).

## PREPARATION FOR ASSAY

1. All reagents should be brought to room temperature (20-25°C) before use. All reagents should be mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Reconstitute each lyophilized standard with 1.0 ml-distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.

## ASSAY PROCEDURE FOR SERUM AND PLASMA

1. Samples of patient serum, plasma and control serum need to be diluted before use for best results. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 10ul serum with 1.0 ml Sample Diluent (101 fold dilution). Do not dilute the standards, they have already been pre-diluted 101 fold.
2. Secure the desired number of coated wells in the holder.
3. Dispense 20ul of standards, diluted specimens, and diluted controls into appropriate wells.
4. Dispense 200ul of Sample Diluent into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix them completely.
6. Incubate at 37°C for 30 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container.
8. Remove liquid from all wells. Wash wells three times with 300ul of 1X wash buffer. Blot on absorbance paper or paper towel.
9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual liquid droplets.
10. Dispense 200ul of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
11. Incubate at 37°C for 30 minutes.
12. Remove the contents and wash the plate as described in step 7, 8, and 9.
13. Dispense 100ul TMB Reagent into each well.
14. Gently mix for 10 seconds.
15. Incubate at room temperature in the dark for 20 minutes.
16. Stop the reaction by adding 100ul of Stop Solution to each well.
17. Gently mix for 10 seconds. It is important to make sure that all the blue color changes to yellow color completely.
18. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

## CALCULATION OF RESULTS FOR SERUM AND PLASMA

1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and patient samples. The standard curve is constructed as follows: against its concentration in .g/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of
2. To construct the standard curve, plot the absorbance for fT4 standards (vertical axis)

## ASSAY PROCEDURE FOR URINE TEST

1. Urine Samples need 10 fold Dilution with the Sample Diluent (i.e. 50ul urine + 450ul Sample Diluentl).
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard

## CALCULATION OF RESULTS FOR URINE TEST

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1. Calculate the mean absorbance value (A450) for each reference standards, controls and patient samples. The standard curve is constructed as follows: against its concentration in .g/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of .2MG in .g/ml. Divide the calculated values by 10.1 (Since the .-2 Microglobulin standards have been prediluted 101 fold, the results obtained from urine samples should be further divided by 10.1). represented. This curve should not be used in lieu of standard curve run with each assay. the real value will be 2.40 .g/ml . 10.1 = 0.238 .g/ml.3.

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