Human 25(OH) Vitamin D ELISA Kit (Competitive EIA)

Catalog number: EK7102

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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Size: 96 wells/kit

Sample Type: Serum and Plasma

Sensitivity: 2.5 ng/ml

Assay Range: 2.5-150 ng/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

This ELISA kit is of competitive format. Competitive ELISA, also known as inhibition ELISA, is a surface/plate based assay, where the plate is coated with capture antibodies reactive to the molecule of interest. The sample (containing native molecule of interest) and enzyme conjugated recombinant protein (the competing molecule) are added to the coated wells. Since the amount of enzyme conjugated molecule in each well is constant, the level of native molecule in the sample will determine the binding ratio of enzyme conjugated molecule vs. native molecule. After an incubation period, any unbound antibody is washed off. Enzyme substrate (for example, TMB for HRP) is added to each well and will be transformed into a blue precipitate, the amount of which is linearly proportional to the amount of enzyme in the well. The precipitate is then turned into yellow by adding the acid stop solution and the concentration of yellow precipitate is read at 450nm for light absorbance (O.D. value). The O.D. is then used to calculate the amount of molecule of interest in each well, by comparing each sample well against the standard curve. The standard curve is generated using the same principle but instead of adding samples, a series of recombinant molecules with known concentrations are added to 6-8 wells.

Kit Components

Description	Quantity
1. Microwell plate coated with anti-Vitamin D	12x8x1
2. Vitamin D Standard Set: 7 vials (ready to use)	0.5 ml
3. Vitamin D Control Set: 2 vials (ready to use)	0.5 ml
4. Biotinylated 25 (OH)D Reagent: 1 Vial (51X)	0.55 ml
5. Assay Diluent, 1 bottle	24 ml
6. Streptavidin-HRP, 1 bottle (ready to use)	23 mL
7. Stop Solution, 1 bottle (ready to use)	12 mL
8. TMB Substrate, 2 bottles (ready to use)	2 x 12 ml
9. Microplate sealing film	2
10. Wash Concentrate 20X, 1 bottle	25 ml

Standard Concentrations and example data

	OD 450 nm	25(OH)D, (ng/ml)
Std 1	2.4	0
Std 2	2.1	2.5

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Std 3	1.76	5
Std 4	1.31	15
Std 5	0.79	35
Std 6	0.48	70
Std 7	0.29	150

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. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory Use.
3. Potential biohazardous materials: The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate
7. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND HANDLING

Serum, heparinized plasma or EDTA plasma samples can be used for the assay. . For serum, collect whole blood by venipuncture and allow clotting. . For plasma, mix the sample by gentle inversion prior to centrifugation. Centrifuge and separate serum or plasma as soon as possible after collection. Do not use hemolyzed samples. Typically, the specimens may be refrigerated at 2-8°C for two weeks. For long term storage, they can be stored at -20°C. Avoid repeated freeze-thaw cycles. Allow the refrigerated or frozen-thawed samples to equilibrate to room temperature for 30 minutes before use; samples must be mixed before analysis.

REAGENT PREPARATION

Before running the test, prepare the following:
1. Standards and Reagents: Standards are serum-based solutions and stable when stored at 2-8°C, protected from light, until the expiration date on

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the label. Equilibrate the needed volume of standards and reagents to room temperature before use.
2. 51X Biotin conjugate: Immediately before use, prepare 1X working solution at 1:51 with assay diluent (e.g. Add 0.1ml of the 51X Vitamin D-Biotin conjugate concentrate to 5ml of assay diluent). Remaining Assay Diluent must be stored at 2-8°C in dark and tightly capped.
3. 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 (20-25°C).

ASSAY PROCEDURE

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be GENTLY mixed without foaming. Once the procedure has started, all steps should be completed without interruption.
1. Dispense 10ul of 25-OH Vitamin D Standards, controls and samples into each well, as required.
2. Dispense 200ul working solution of biotinylated 25 (OH) D reagents, into each well.
3. Carefully mix the contents in the wells for 20 seconds using a plate shaker at 200-400 RPM (or equivalent motion). Remove from shaker and cover the plate with the adhesive plate seal making sure there is a complete seal over each well.
4. INCUBATION #1 -- Incubate sealed plate for 90 minutes at room temperature.
5. Carefully remove the plate seal.
6. Briskly shake out the contents of the wells into a waste reservoir.
7. WASH # 1 - Dispense 300ul of 1X Wash Buffer into each well, and then briskly shake out the 1X Wash Buffer into a waste reservoir. Strike the wells sharply on absorbent paper to remove residual droplets. Repeat 2 more times for a total of 3 washes.
8. Dispense 200ul of enzyme conjugate (Streptavidin-HRP) into each well.
9. INCUBATION #2 - Incubate for 30 minutes, at room temperature.
10. Briskly shake out the contents of the wells into a waste reservoir.
11. WASH # 2 - Dispense 300ul of 1X Wash Buffer into each well, and then briskly shake out the 1X Wash Buffer into a waste reservoir. Strike the wells sharply on absorbent paper to remove residual droplets. Repeat 2 more times for a total of 3 washes.
12. Using a multi-channel pipette, dispense 200 ul of TMB Substrate into each well.
13. INCUBATION #3 - Incubate for 30 minutes at room temperature, preferably in the dark.
14. STOP - Dispense 50 ul of Stop Solution into each well to stop the enzymatic reaction. Carefully mix plate contents for 20 - 30 seconds.
15. Read absorbance on ELISA Reader at 450 nm within 10 minutes of adding the Stop Solution. 050100150200012325 (OH)D ng/mLOD450nm

QUALITY CONTROL

We recommend that each laboratory uses 25-OH Vitamin D controls to validate the performance of reagents. RESULTS Results are expressed in ng/mL. Note: To convert to nmol/L, multiply results by 2.5. Example: 10ng/ml = 25nmol/L.

REFERENCE RANGE

It is recommended that each laboratory establishes the range of normal values that corresponds to the population of their region.

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