Size : 137 x 218 mm

Qualisa™
Microwell Enzyme Immunoassay | VIT D

25-OH Vitamin D

Enzyme linked Immunosorbent Assay (ELISA) for Quantitative Determination of total 25-OH Vitamin D in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Qualisa™ 25-OH Vitamin D Sandwich ELISA test is intended for the quantitative determination of total 25-hydroxy (25-OH) Vitamin D in human serum. For InVitro Diagnostic Use only.

INTRODUCTION

Vitamin D is a steroid hormone responsible for enhancing intestinal absorption of calcium and the regulation of its homeostasis. There are two common forms of Vitamin D: Vitamin D_2 and D_3. Vitamin D_3 is naturally produced in the human skin through the exposure to ultraviolet light and Vitamin D_2 is mainly obtained from plant foods. Vitamin D is transported to the liver where it is metabolized to 25-hydroxy Vitamin D. In medicine, a total 25-hydroxy Vitamin D test is used to determine Vitamin D concentration in the body. The blood concentration of 25-hydroxy Vitamin D (including D_2 and D_3) is considered the best indicator of Vitamin D status.

PRINCIPLE OF THE ASSAY

Qualisa™ 25-OH Vitamin D Quantitative Test Kit is a sandwich-based enzyme-linked immunosorbent assay. The test employs a pair of monoclonal agglutinating sera, first one is immobilized on solid phase (Microwells) and another monoclonal agglutinating sera is in the liquid phase. In the assay procedure, samples along with Calibrators are added to the coated Microwells & incubated together with the first & second agglutinating sera. The wells are then washed to remove the unbound components. The resulted Vitamin D-antibody immunocomplex is detected with a third agglutinating sera conjugated with horseradish peroxidase (HRPO). After a short incubation the wells are washed again and bound enzyme is detected by adding substrate. The reaction is stopped after specified time with stop solution and absorbance is determined for each well using an ELISA reader.

The concentration of Vitamin D is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

A. Materials provided with the test kit

1. Coated Microwells: Microwells coated with monoclonal anti-25-OH Vitamin D – antibody.
2. Vitamin D Sample Diluent. Ready to use.
3. Vitamin D Enzyme Conjugate. Ready to use.
4. TMB Substrate. Ready to use
5. Stop Solution. Ready to use
6. Two levels of controls (Control values are provided in the kit)
7. 25-OH Vitamin D Calibrator set of 6 Calibrators labeled as A to F in liquid form. Ready to use. For calibrator concentration refer vial label.
8. Wash Buffer Concentrate (20x).

B. Materials required but not provided

1. Precision pipettes: 10µl, 50-200µl, 100-1000µl
2. Disposable pipette tips
3. Distilled water
4. Disposable Gloves
5. ELISA reader
6. ELISA washer

STORAGE AND STABILITY

1. **Qualisa™ 25-OH Vitamin D** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to white at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable upto one week when stored at 2-8°C.

SAMPLE COLLECTION

1. Collect Blood specimen by venipuncture according to Calibrators procedure.
2. Serum only should be used.

3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing particulate matter should be clarified by centrifugation prior to use.

PRECAUTIONS

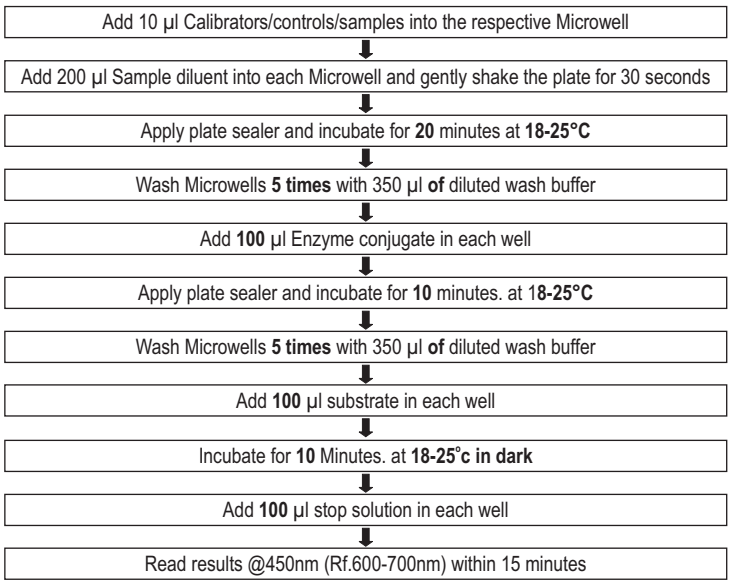
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. Dispose off all the reagents and material used as if they contain infectious agent.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do NOT induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water) Mix well before use.

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 10 µl of calibrator, serum, and controls into the appropriate wells.
2. Dispense 200 µl of Sample Diluent into each well. Gently shake the plate to mix the contents. Incubate at room temperature (18-25°C), for 20 minutes.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer (20X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
4. Dispense 100 µl of enzyme conjugate reagent into each well. Incubate at room temperature(18-25°C) for 10 mins.
5. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer (20X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
6. Dispense 100 µl of TMB substrate into each well. Incubate at room temperature (18-25°C), in the dark, for 10 minutes.
7. Stop the reaction by adding 100 µl of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
8. Read the optical density at 450/630 nm with a microtiter plate reader within 15 minutes.



CALCULATION OF RESULTS

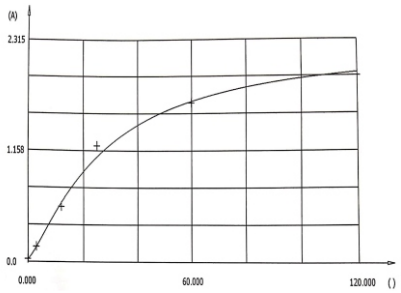
Construct a Calibrator curve by plotting the absorbance obtained from each reference Calibrators against its concentration in units per ml on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of 25-OH Vitamin D in units per ml from the Calibrators curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Calibrator curve

Results of a typical Calibrator run with optical density reading at 450nm (ref 600-700nm) shown in the Y axis against 25-OH Vitamin D concentrations shown in the X axis.

Suggest: Use 4-Parameter Calibrator curve to calculate sample values.

Vitamin D Values (ng/ml)	Absorbance
A	0.011
B	0.053
C	0.210
D	0.437
E	1.006
F	1.928



This Calibrator curve is for the purpose of illustration only, and should not be used to calculate samples. Each user should obtain his or her own Calibrators curve and data.

Expected Ranges of values

Multiple guidelines for Vitamin D deficiency have been published. Recent literature has suggested the following ranges for the classification of Vitamin D status:

25-OH Vitamin D Level	Reference Range (ng/ml)
Deficient	0 – 10
Insufficient	10 – 30
Sufficient	30 – 100
Toxicity	>100

PERFORMANCE CHARACTERISTICS

External Evaluation:

Qualisa™ 25-OH Vitamin D is evaluated by a NABL accredited lab against their reference method (ELISA). In this evaluation **Qualisa™ 25-OH Vitamin D** ELISA demonstrated 100% correlation with the reference method (ELISA).

*Data on file: Qualpro Diagnostics (A Division of Tulip Diagnostics Private Limited)

Internal Evaluation

In an internal evaluation, 300 random samples collected from local lab were evaluated against a reference method (ELISA) & following is our observations:

Total samples = 300	Qualisa™ 25-OH Vitamin D	Reference Method
Deficient	26	29
Insufficient	239	236
Sufficient	34	34
Toxicity	1	1

On the basis of the above evaluation data **Qualisa™ 25-OH Vitamin D** has demonstrated 99.0% correlation with reference method.

16 samples whose LC-MS/MS values have been obtained by a reference lab when tested with **Qualisa™ 25-OH Vitamin D** and following are the results

Total Samples -16	Qualisa™ 25-OH Vitamin D	Reference Method
Deficient	1	1
Insufficient	12	13
Sufficient	3	2
Toxicity	0	0

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multiple channel pippets to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of Calibrators & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. The results obtained from this assay are not diagnostic proof of the presence or absence of a disease.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

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