Diagnostic Kit for 25-hydroxy Vitamin D
(Fluorescence Immunochromatographic Assay)
Instructions for Use

INTENDED USE

This kit is intended for in vitro quantitative detection of 25-hydroxy Vitamin D (25-OH Vitamin D) in human serum/fresh finger terminal blood samples to evaluate the level of Vitamin D. The kit only provides test result of 25-hydroxy Vitamin D. The obtained result shall be analyzed in combination with other clinical information. It must only be used by healthcare professionals.

SUMMARY

Vitamin D is also a steroid hormone. It mainly includes VD_2 and VD_3, which have very similar structure. Vitamin D_3 and D_2 are carried through blood circulation into the liver and converted into 25-hydroxy Vitamin D (including 25-dihydroxyl Vitamin D_3 and D_2) by the effect of Vitamin D-25-hydroxylase. 25-hydroxy Vitamin D is mainly converted into physiologically active 1, 25- dihydroxyl Vitamin D in the kidney under catalysis of 25OH-1α hydroxylase. 25-(OH)VD exists in human body in high concentration and stably, and can reflect the total amount of Vitamin D ingested from food and synthesized by the body as well as the conversion ability of Vitamin D. Therefore, 25-(OH)VD is considered as the best indicator for evaluating nutritional status of Vitamin D.

PRINCIPLE OF DETECTION

This kit uses fluorescence immunochromatographic analysis with highly specific dual-antibody sandwich immunoassay reaction principle to quantify the concentration of 25-(OH)VD in human serum/fresh finger terminal blood samples. The test area (T) of the nitrocellulose membrane is coated with anti-25(OH)VD antibody and antigen complex antibody. The QC region (C) with goat anti-rabbit IgG polyclonal antibodies. The labeling pad contained pre-coated fluorescently labeled mouse anti-25-(OH)VD-conjugated monoclonal antibody and rabbit IgG polyclonal antibodies. During detection, the 25-(OH)VD in the sample is first separated from the vitamin D-binding protein with the sample treatment solution, and the free 25-(OH)VD will first combine with the fluorescently labeled mouse anti-25-(OH)VD-labeled monoclonal antibodies on the labeling pad to form an immune complex, which flows with the unbound fluorescent marker along the sample pad and the inside of the nitrocellulose membrane to the absorbent paper under the action of chromatography. The immune complex binds to the anti-25(OH)VD antibody coated on the membrane and the antigen complex antibody when it passes through the test region (T), and when it passes through the QC region (C), the fluorescently labeled rabbit IgG polyclonal antibodies binds to the coated goat anti-rabbit IgG polyclonal antibodies to form a "sheep anti-rabbit IgG polyclonal antibody-fluorescently labeled rabbit IgG polyclonal antibody" complex and agglutinates. The concentration of 25-(OH)VD in the sample is positively correlated with the fluorescence signal, and the concentration of 25-(OH)VD in the sample is calculated by the immunoassay analyzer according to the relative fluorescence signal intensity detected.

为正生物 WIZBIOTECH
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MAIN KIT COMPONENTS

Catalogue number	53321001	53321005	53321020	53321025
specification / Components	1 Test/Kit	5 Tests/Kit	20 Tests/Kit	25 Tests/Kit
Test Device(s)	1	5	20	25
Sample Diluents	1	5	20	25
Instructions for Use	1	1	1	1

MAIN ACTIVE INGREDIENTS

1. Test line (T line): T line area of nitrocellulose membrane is coated with conjugate of 25-(OH)VD and Antigen complex antibodies.
2. Control line (C line): C line area of nitrocellulose membrane is coated with goat anti-rabbit IgG antibody.
3. Sample Processing: Mainly contains Tris-HCl buffer, Tween-20, etc

Warning: The diluent includes 0.05% Proclin300

H317: May cause an allergic skin reaction.

H412: Harmful to aquatic life with long lasting effects.

P280: Wear protective gloves/protective clothing/face protection.

P333+P313: If skin irritation or a rash occurs: Get medical advice/attention.

P362+P364: Take off contaminated clothing and wash it before reuse.

4. marker pad: Contains fluorescent bead-conjugated murine anti-25-(OH)VD-conjugated monoclonal antibodies and rabbit IgG polyclonal antibodies.

STORAGE CONDITION

1. The kit should be stored at 2℃~30℃. The shell life of the kit is 24 months.
2. Do not use the kit after the expiration date

APPLICABLE INSTRUMENT

The test must be quantified with Igloo Reader Pro, available from goodscare GmbH, Germany.

SAMPLE COLLECTION AND STORAGE

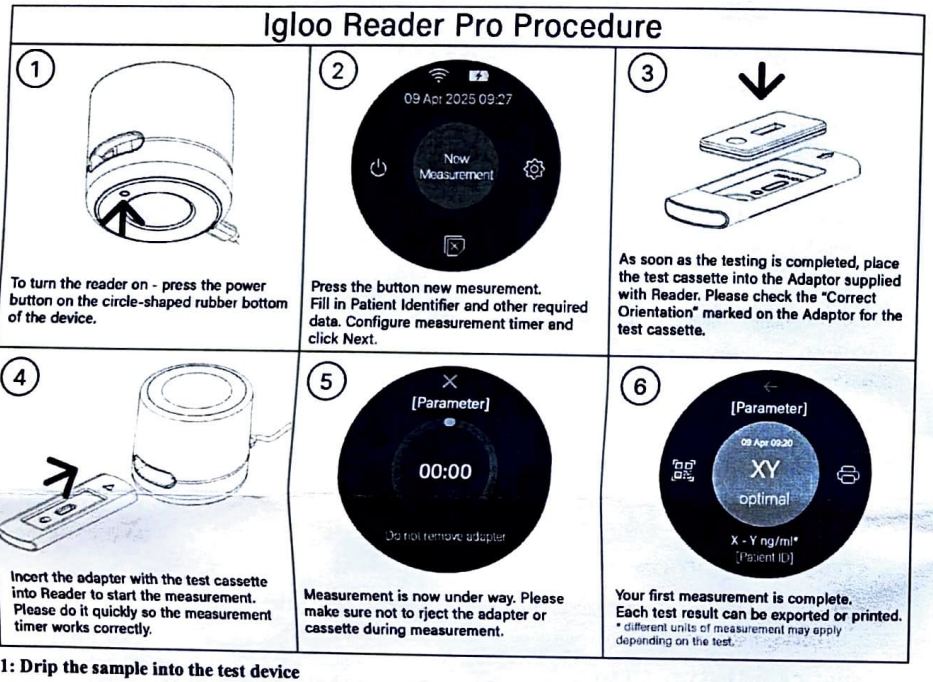
1. The samples tested can be serum, and fresh finger terminal blood.
2. Serum sample collection: refer to the venous blood collection method of the "National Clinical Laboratory Operating Procedures", if the test cannot be done in time, the sample can be stored in the refrigerator at 2~8℃, and should be frozen at -15℃ for more than 1 day, and can be stored at -15℃ for 6 months.
3. Fresh terminal blood of fingertips should be used immediately after collection.
4. The sample needs to be returned to room temperature before testing.

REAGENT PREPARATION

1. Use immediately after open the aluminum foil bag.
2. Before test, restore the reagent to room temperature.

TEST METHOD

Read the instruction for use and test operation manual completely before the test and restore the reagent to room temperature before the test. Do not perform the test without restoring the reagent to room temperature to avoid affecting the accuracy of the test results.



1: Drip the sample into the test device

(1) Open the aluminum foil bag package, take out the test device, and horizontally place it on examination table;

(2) Slowly and one-time draw 10 μ L of serum/fresh finger terminal blood sample, add it to the sample processing solution, and mix well for 1min;

(3) Add 80μL of the above mixed solution to the sampling hole of the detection card, and be careful not to inhale air bubbles when sampling;

(4) Reaction time is 15 minutes.

REFERENCE INTERVAL

1. Study of 25-(OH) VD reference interval is conducted through referring to C28-A2 document published by US Clinical and Laboratory Standards Institute (CLSI)- How to Define and Determine Reference Intervals in the Clinical Laboratory-Second Edition and WST 402-2012 Define and Determine the Reference Intervals of Test Items in Clinical Laboratory. The obtained reference interval of 25-(OH) VD is: 30-100ng/mL.
2. Due to difference in geography, race, age etc., each laboratory is suggested to establish reference interval of 25-(OH)VD that is suitable for local populations and has clinical significance.

INTERPRETATION OF THE RESULT

1 If the measured 25-(OH)VD concentration of the sample is higher than the range of reference value, state such as physiological change or stress reaction should be excluded. It is indeed abnormal and should be diagnosed in combination with clinical symptoms. Above result is only for reference.

2 Test result of this method is only applicable to evaluation using the reference value established in this method and cannot be directly compared with result of other method.

3 Other factors that may cause wrong test result include technical reason, operation error and other sample factors.

*Invalid result: If the assay result is invalid, the Igloo Reader Pro will display an "invalid" result. The test personnel should read the kit instructions and portable immunoassay analyzer instructions carefully and repeat the test. If the "invalid" result is obtained If it reappears, please contact the device manufacturer.

PRECAUTIONS

1. This kit can only be used for in vitro diagnosis.
2. This kit is for healthcare professionals only.
3. Before test, restore reagent and sample to room temperature.
4. This kit is disposable.
5. Do not use expired reagent.
6. Sample collection and storage must be performed in strict accordance with this instruction.
7. The reagent should be stored in strict accordance with the conditions specified in this instruction for use. Do not store the reagent under freezing condition.
8. Do not open the aluminum foil bags before test and protect the products from moisture; do not use if the aluminum foil bags are damaged or if the test reagents are wet.
9. For all components of the kit, it is recommended not to mix or interchange different batches.
10. Excessive or insufficient sample may lead to deviation of result.
11. Do not confuse sample hole with result observation window. Adding sample to result observation window will make test result invalid.
12. Test method should strictly follow the instruction for use.
13. For specific explanation of test result, analysis shall be performed in combination with clinical information.
14. The used reagent and sample shall be properly disposed as medical waste with risk of biological infection and handled safely.
15. Desiccant in aluminum foil bag is inedible.
16. Sample diluents is only used for test. Do not drink it. Wrong use may lead to biological hazard.
17. During test, test procedure, precautions and result explanation of the reagent must be followed to avoid wrong result.

PRODUCT PERFORMANCE INDEX

Evaluation performed using internal enterprise reference, that all performance indexes of this kit conform to standard. Specific performance indexes are as below:

1. Reportable range of the reagent (kit) is 5ng/mL~200ng/mL.
2. Linear range: Linearity of the reagent (kit) is within 5ng/mL~120ng/mL. Analysis performance meets the following requirement:

a) The correlation coefficient (r) of linearity should be≥0.9900.

3. Accuracy: The recovery rate should be within the range of [85% ~ 115%].
4. Limit of detection: ≤3ng/mL.

5. Repeatability: CV≤15%.

6. HOOK effect: When concentration of 25-(OH) VD is 400ng/mL, there is no Hook effect.

7. Specificity: No cross-reaction phenomenon occurs when the following substances are tested under the shown concentration condition.

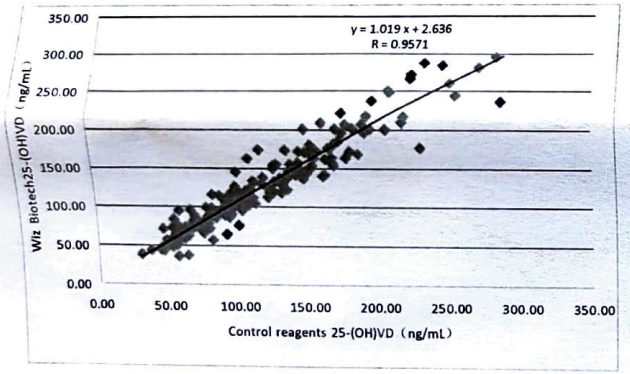
Material	Concentration	Material	Concentration
Vitamin D3	50 mg /mL	Calciferol	50 mg /mL

8. Interfering substance: Following substances are found to cause no interference when they are tested at the given concentration.

Interfering substance	Concentration	Interfering substance	Concentration
Bilirubin	2.0 mg/mL	Triglyceride	40.0 mg/mL
Hemoglobin	10.0 mg/mL	Rheumatoid factor	1500.0IU/mL

9. Clinical performance

Clinical evaluation performance of the product is assessed through collecting 196 clinical samples. The results are compared by using the corresponding kit of marketed chemiluminescence kit as the reference reagent. Use linear regression to investigate their comparability. Use linear regress to investigate their comparability. The correlation coefficients of the two tests are Y=1.019X+2.636 and R=0.9571, respectively.



LIMITATION

1. This reagent is only used for testing human serum, and fresh finger terminal blood.
2. Do not agitate the sample. Insert a pipette just below the surface of the sample to collect the specimen.
3. Test shall be carried out at normal room temperature (18℃~25℃).
4. Linearity range of the kit is 5ng/mL～120ng/mL . To measure accurate concentration of high-concentration sample that exceeds measurement linearity range of the kit, measurement and calculation should be performed after dilution to within the linearity range of the kit. Under the conditions of normal saline or sample diluents, the maximum dilution multiple of this kit about 1.5 time and the reportable range is: 5ng/mL~200 ng/mL.
5. Due to low concentration of the analyte, this method cannot detect analyte, this will lead to result deviation.
6. Since some non-specific reactions or other cross reactions cannot be fully studied, false positive results may occur in this test.
7. This test has a low probability of false positive results. Therefore, all positive results must be verified by other method.

8. If the obtained result is questionable, immediately re-test or use other method to test the sample.
9. Test result of this reagent can only be used as an auxiliary means for doctor or other diagnosis. Test result should be combined with other clinical and laboratory data. If test result is not consistent with clinical evaluation, further examination will be required.

LITERATURE REFERENCES

[1] Jukic AMZ, Hoofnagle AN, Lutsey PL. Measurement of Vitamin D for Epidemiologic and Clinical Research: Shining Light on a Complex Decision. Am J Epidemiol. 2018;187(4):879-890.
[2] Charoenngam N, Holick MF. Immunologic Effects of Vitamin D on Human Health and Disease. Nutrients. 2020;12(7):2097. Published 2020 Jul 15.
[3] National Osteoporosis Foundation Prevention-Vitamin D.

SYMBOLS

Symbol	interpretation	Symbol	interpretation	Symbol	interpretation
	Consult instructions for use		Tests per kit		Manufacturer
IVD	In Vitro Diagnostic Medical Device		Use-by date		Do not re-use
30℃ 2℃	Store at 2℃~30℃	REF	Catalogue number	LOT	Batch code
EC REP	Authorized Representative in the European Community		Caution		

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