Instruction for 25-OH Vitamin D (25-OH Vit-D) Test Kit (Immunofluorescence)

1. PRODUCT NAME

Generic name: 25-OH Vitamin D (25-OH Vit-D) Test Kit (Immunofluorescence)

Trade name: 25-OH Vit-D.

2. PACKAGE

Specification 1: 25T/kit REF: 52026116

Specification 2: 50T/kit REF: 52027111

Quality Control (optional):

Level 1: 0.5mL × 1 REF: 52105084

Level 2: 0.5mL × 1 REF: 52105085

Level 3: 0.5mL × 1 REF: 52105086

3. INTENDED USE & INDICATION

For in vitro quantitative determination of 25-OH Vit-D level in human serum, plasma or whole blood or capillary blood. Clinically, it is mainly used for auxiliary diagnosis of diseases related to vitamin D deficiency.

For professional use only.

4. TEST PRINCIPLE

This kit adopts the competition method. After the samples containing 25-OH Vit-D are fully mixed with the sample diluent, 25-OH Vit-D in the sample is released from vitamin D binding protein, an appropriate amount of the solution is taken and added into the sample well. The 25-OH Vit-D in the sample binds to the mouse anti-25-OH Vit-D monoclonal antibody coupled to the fluorescent particles to form a fluorescent particle-antibody-antigen complex. This immune complex is then chromatographed along the nitrocellulose membrane to the determination area (T). The unbound fluorescent particle-antibody conjugate binds to the precoated 25-OH Vit-D-conjugated antigen and continues to chromatograph to the quality control area (C). The fluorescent particle-labeled goat anti-rabbit IgG binds to the precoated rabbit IgG and presents the quality control line. The fluorescence intensity of the assay area is inversely proportional to the level of 25-OH Vit-D in the sample.

5. MAIN COMPONENTS & ADDITIONAL REQUIRED EQUIPMENT

The test kit consists of test card, magcard, sample diluent, quality control (optional) and the instruction.

(1) The test card consists of card shell and test strip. The test strip contains sample pad/marking pad, nitrocellulose membrane, absorbent paper and PVC plate.

(2) Magcard: load calibration curve information for this batch of reagents.

(3) Sample diluent: the main ingredient is phosphate buffer (PBS). It is portioned into 0.5 mL per tube for each test.

(4) Quality control (optional): Self-prepared lyophilized powders, mainly consist of 25-OH Vit-D recombinant antigen and PBS. All are free of human-derived substances and have batch specificity. Please find target values in the target value list.

(5) Equipment: applicable to FA50 and FA120 Quantitative Immunoassay Analyzer manufactured by Genrui Biotech Inc.

Note: Components of kits from different batches should not be used interchangeably.

6. ACCESSORIES REQUIRED BUT NOT PROVIDED

(1) Pipettes and pipette tips: 100 μL

(2) Timer

7. SPECIAL STORAGE & TRANSPORT CONDITIONS

(1) The test kit should be stored at 2-30℃, and the shelf life of test cards and sample diluent is 18 months when sealed. After the test card and sample diluent are opened, the shelf life is 1 hour at 18-30℃ and 40%-65% humidity. When the humidity is > 65%, it should be used right after opened.

(2) The unopened QC is stable for 18 months (see the label for specific date) at -25℃ to 8℃,the reconstituted QC is stable for 6 days at -20℃ or 1 day at 2-8℃ in the shade, and can be freeze-thawed once.

(3) Transport: The test kit is at 2-30℃, the QC is at -25℃-8℃.

8. SAMPLE REQUIREMENTS

(1) The optimal sample is fresh non-hemolyzed serum, plasma or whole blood or capillary blood. It is recommended to use sample from venous blood, as results of other body fluids and samples may not be accurate.

(2) The capillary blood should be tested immediately.

(3) Serum/plasma: After sample collection, serum should be separated as soon as possible to avoid hemolysis. Serum and plasma should complete the test within 6 hours at room temperature. The samples that cannot complete the test should be refrigerated at 2-8℃ for no more than 7 days; serum and plasma should be frozen below -18℃ for no more than 1 month.

(4) Whole blood: It should be used immediately after collection. If it cannot be tested within 4 hours, it should be refrigerated at 2-8℃ for no more than 3 days. Samples should not be frozen.

(5) The samples should be brought to room temperature before determination. The frozen samples should be completely thawed, rewarmed and mixed well before use. Do not freeze and thaw repeatedly.

(6) Human serum is preferred for determination, and sodium citrate or EDTA-K_2 is recommended as an anticoagulant for plasma and whole blood testing.

9. TEST METHOD

Carefully read the instruction before using the test kit and operate in strict accordance with the instruction to ensure reliable results. Bring all reagents to room temperature (18-30℃) before use.

(1) Startup: Click “STD Mode” in the main menu to enter the measurement interface, click “Item” to select the test item and click “Type” to select the sample type.

(2) Click “Lot No.” to enter the card reading interface, place magcard of the corresponding item to the magnetic card reader area, when the magcard is read successfully, check whether the magcard and the test card are of the same batch. (Note: reagents are precalibrated and specific calibration curve parameters for each batch of reagents have been stored in the magcard.)

(3) Quality control procedure: It is recommended to refer to the instrument manual and use the Genrui quality control to verify whether the target value of the test quality control is under control during the measurement procedure after calibration. The quality controls should be used as follows.

a）Bring the quality control to room temperature (18-30°C) before use.

b）Carefully open the bottle cap to avoid spraying of the contents.

c）Add 0.5 mL of purified water.

d）Put on the bottle cap and leave it at room temperature for 15 minutes, gently shake the bottle to fully dissolve the dry powder.

e）After the dry powder is fully dissolved, repeat the operation for the sampling.

If the measured values of quality controls are within the given range of target values, the determination of clinical samples and data analysis can be continued; otherwise, the causes should be identified before test.

(4) Sampling:

①Add 0.1 mL of plasma, serum or whole blood into the container with 0.5mL of sample diluent, mix thoroughly and leave it for 15 minutes. Take 0.1mL of diluted sample, and drop it vertically to the sample well on the test card directly and start timing.

②Add 0.04mL of capillary blood into the container with 0.2mL of sample diluent, mix thoroughly and leave it for 15 minutes. Take 0.1mL of diluted sample, and drop it vertically to the sample well on the test card directly and start timing.

(5) Insert it into the analyzer’s test card slot (the sample well end towards the inside). Click “Measure”, the instrument will automatically detect and print out the results after 15 minutes (If using “Fast Mode”, after 15 minutes of external incubation, quickly insert

card and click “Measure”, then instantly the instrument will detect and print out the results).

Note: For detailed instructions on how to operate the instrument, please refer to the manual of Quantitative Immunoassay Analyzer.

10. REFERENCE RANGE

Reference range: ≥30ng/mL

Note:2.5 × ng/mL = nmol/L

Due to geographical, ethnic, gender and age differences, it is recommended that each laboratory establishes its own reference range.

11. EXPLANATION FOR TEST RESULTS

(1) When the control area (C) appears fluorescent strips, the analyzer will automatically detect the fluorescence and analyze the test card, and then provide quantitative results.

(2) When the control area (C) does not appear fluorescent strips, the analyzer cannot detect the fluorescence and alarm will be activated automatically, indicating that the operation is incorrect or the test card is damaged. In this case, carefully read the instructions again and re-test with a new test card, if the problem still exists, immediately stop using products of this batch and contact your supplier.

(3) When the measured value of the sample is higher than 100 ng/mL, the instrument shows > 100 ng/mL, and when the measured value of the sample is less than 5 ng/mL, the instrument shows < 5 ng/mL.

12. DETECTION LIMIT

(1) This test kit is for in vitro diagnostic use only.

(2) Diagnosis and treatment can not solely base on this test result, please taking into account the clinical history and other laboratory test results. Each laboratory is recommended to establish its own reference range based on its patient population.

13. INTERFERING SUBSTANCE

(1) Hemoglobin, bilirubin, cholesterol, triglyceride, total protein, HAMA and rheumatoid factor in samples can interfere with the test results, the maximum allowable concentrations of hemoglobin is 2 g/dL, bilirubin is 66 mg/dL, cholesterol is 1000 mg/dL, triglyceride is 5000 mg/dL, total protein is 500 g/L, HAMA is 1000 ng/mL, rheumatoid factor is 800 IU/ mL.

(2) Potential cross-reactants are as follows:

cross-reactant	cross-reactant concentration	cross-reactivity (%)
25-OH-Vit-D_3	20~60ng/mL	100.7
25-OH-Vit-D_2	34.50~49.12ng/mL	100.0
24，25-（OH）$_2$-Vit-D_3	20ng/mL	105.3~133.3
C-3-epimer of 25-OH-Vit-D_3	100ng/mL	1.3
1，25-（OH）$_2$-Vit-D_3	100ng/mL	0.7
1，25-（OH）$_2$-Vit-D_2	100ng/mL	0.3
Vit-D_3	100ng/mL	0.6
Vit-D_2	100ng/mL	0.4

14. PRODUCT PERFORMANCE INDICATORS

(1) Limit of detection: ≤ 5 ng/mL

(2) Linearity range: 5-100 ng/mL (Linear correlation coefficient: r ≥ 0.9900)

(3) Precision: intra-batch precision: CV ≤ 15%; inter-batch precision of the kit CV ≤ 15%

(4) Accuracy: -15% ≤ Bias% ≤ +15%

(5) QC precision: CV ≤ 15%

(6) Expected results of QC: the test results shall be within the target range

(7) Moisture content: the moisture content of the QC (lyophilized powder) is ≤ 10%

15. PRECAUTIONS

(1) Once opened, use the test cards as soon as possible, which may be exposed to moisture in the air. Do not reuse the test cards.

(2) Components in test kit of different batches cannot be used interchangeably.

(3) For substances containing sources of infection or suspected of containing sources of infection, there should have proper bio-safety assurance procedures. Pay attention to the following notes:

-- Wear gloves when handling sample or disinfecting the reagent.

-- Disinfect spilled sample or reagent with disinfectant.

-- Disinfect or handle potential contamination sources of all samples or reagents in accordance with local regulations.

16. EXPLANATION OF GRAPHIC SYMBOL

	Consult instructions for use		Temperature limit
LOT	Batch code		Use-by date
IVD	*In vitro* diagnostic medical device	CE	CE Marking
	Date of manufacture		Biological risks
	Manufacturer		Volume
Σ	Contains sufficient for < n>tests		Keep away from sunlight
	Do not re-use		Keep dry
EC REP	Authorized representative in the European community	REF	Catalogue number

17. REFERENCE

(1) Hart GR, etal. Measurement of vitamin D Status: background, clinical use and methodologies.Clin Lab 2006;52(7-8):335-343.

(2) Venning G.Recent developments in vitamin D deficiency and muscle weakness among elderly people .BMJ 2005;330:524-526.

(3) Kuchukk NO,van Schoor NM, Pluijm SM, et al.Vitamin D status,parathyroid function, bone turnover, and BMD in postmenopausal women with osteoporosis: global perspective.J Bone Miner Res 2009;24:693-701.

18. METROLOGICAL TRACEABILITY

The kit is traceable to the LC-MS/MS reference measurement procedure.

19. HELP INFORMATION

If you need help, please contact after sales department.

20. MANUFACTURER

Genrui Biotech Inc.

Address: 4-10F, Building 3, Geya Technology Park, Guangming District, 518106, Shenzhen, China

21. INSTRUMENTS & APPLICATIONS

Genrui’s Immunofluorescence products are designed to work in automated lab, which are compatible with the FA50/FA120 Quantitative Immunoassay Analyzer. There may or may not be an application developed for your particular instrument, please visit the instrument section of our website.

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