Instruction For Use

【Product Name】

25-OH Vitamin D Total (Electrochemiluminescence Immunoassay)

【Order Information】

REF NO.	Package Size
693011	100T
693025	2×100T
693021	50T
693024	2×50T

【Intended Use】

Immunoassay for in vitro quantitative determination of Total 25-hydroxy Vitamin D in human serum and plasma. The results can be used in assisting diagnosis of Vitamin D sufficiency.

Vitamin D is a fat-soluble steroid hormone precursor that is essential for the body to promote calcium absorption in the intestine and to regulate calcium balance. To form and maintain a strong and healthy bone, vitamin D is essential. The level of vitamin D in the body is affected by many factors, including ultraviolet radiation, dietary supplementation, age, obesity, ethnicity and ethnic factors. Vitamin D itself is not biologically active and must undergo two successive hydroxylation reactions to be converted into a biologically active form. The first hydroxylation occurs in the liver, producing 25-hydroxyvitamin D, and the second hydroxylation occurs primarily in the kidneys, producing the biologically active metabolite 1, 25-dihydroxyvitamin D. When 1, 25-dihydroxyvitamin D is sufficient, it can be further converted to 24, 25-dihydroxy- vitamin D in the kidney[1]. Two most important forms of vitamin D are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocallciferol). Human can only produce D_3 vitamins or calciferols by the action of ultraviolet rays from sunlight on the skin. In contrast to vitamin D_3, the human body cannot pruduce Vitamin D_2 which is taken up with fortified food or given by supplements[2-3]. In human serum and plasma, vitamin D_3 and Vitamin D_2 are bound to the vitamin D Binidng Protein and transported to the liver. Epidemiological studies have shown a high global prevalence of Vitamin D insufficiency and deficiency[4]. Vitamin D deficiency causes bone metabolism weakness such as rickets and osteoporosis. Therefore, 25-OH Vitamin D is the analyte of choice for determination of the vitamin D status. The measurement of vitamin D status provides opportunities for preventive and therapeutic interventions[5].

【Test Principle】

Competition principle. Total duration of assay: 27 minutes

1st incubation: By incubating the sample (30 µL) with pretreatment reagent 2 and 3, bound 25-OH Vitamin D is released from the vitamin D binding protein.

2nd incubation: By incubating the pretreated sample with the ruthenium labeled monoclonal vitamin D-specific antibody, a complex between the 25-OH vitamin D and the ruthenylated vitamin D monoclonal antibody is formed.

3rd incubation: After addition of streptavidin-coated microparticles and 25-OH vitamin D labeled with biotin, unbound ruthenium labeled vitamin D monoclonal antibody become occupied. A complex consisting of the ruthenylated vitamin D monoclonal antibody and the biotinylated 25-OH vitamin D is formed.

After incubation, The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with system reagent. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the RFID.

【Main Components】

The reagent pack consists of MB, RA, RB, PT2, PT3, calibrators and control materials (Optional). Different lots cannot be used at the same time or mixed up together.

Components	Ingredients	Content (2×50T)	Content (50T)	Content (100T)	Content (2×100T)
Pretreatment reagent 2 (PT2)	1.4% Dithiothreitol	2×3.0 mL	1×3.0 mL	1×4.0 mL	2×4.0 mL
Pretreatment reagent 3 (PT3)	0.25M Sodium Hydroxide	2×2.5 mL	1×2.5 mL	1×3.0 mL	2×3.0 mL
Magnetic Beads (MB)	Streptavidin-coated microparticles, 0.75 mg/mL; 100 mM PBS; 0.05% ProClin™ 300	2×3.0 mL	1×3.0 mL	1×5.0 mL	2×5.0 mL
Reagent B (RB)	Biotinylated 25-OH Vitamin D, 1.5 mg/L; 50 mM BIS-Tris; 0.05% ProClin™ 300	2×5.0 mL	1×5.0 mL	1×8.0 mL	2×8.0 mL
Reagent A (RA)	Ru complex-labeled 25-OH Vitamin D antibody, 1.2 mg/L; 50 mM BIS-Tris; 0.05% ProClin™ 300	2×5.5 mL	1×5.5 mL	1×9.0 mL	2×9.0 mL
25-OH VD Calibrators	25-OH Vitamin D antigen; Horse serum, 0.05% ProClin™ 300; lyophilized	High: 1×1.0 mL Low: 1×1.0 mL			
25-OH VD Control Materials (Optional)	25-OH Vitamin D antigen; Horse serum, 0.05% ProClin™ 300; lyophilized	High: 1×1.0 mL Low: 1×1.0 mL			

This method has been traceable to the National Institute of Standards and Technology Standard Reference Material 972a.

See the control material value sheet for the target values and ranges of control materials.

Additional materials for Automated ECL Immunoassay Analyzer eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, o REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Storage and Shelf Life】

Store at 2ºC-8ºC.

Do not freeze.

Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagents	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks
on the analyzer at 2ºC~15ºC	8 weeks

The calibrators and control materials are stable up to the stated expiration date.

Stability of the calibrators and control materials	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after resolution	5 days
Store at -25ºC~-15ºC after resolution	3 months (freeze only once)

The expiration date is labeled on the box, rackpack and bottles.

Damaged, expired or contaminated reagents should be discarded.

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

Human serum and plasma added with heparin lithium, EDTA-K_3 and EDTA-K_2 anti-coagulants are recommended. Blood samples should be collected by standard operation of venous puncture, after complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not recommended.

Samples can be stored for 8 hours at 18ºC~28ºC, 4 days at 2ºC~8ºC, and 6 months at -25ºC~-15ºC. The samples can be freeze-thawed only once.

The samples should be centrifugated before test if samples are deposited and frozen. Taking into account the possible factor of evaporation, the test of samples, calibrators and control materials is performed as short as possible within 2 hours.

【Assay Procedure】

Testing procedures and precautions

Previous to operation, one should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up 25-OH vitamin D test according to the operational manual.

Put the 25-OH vitamin D rackpack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.

The sample volume required for each 25-OH vitamin D test is 30 µL.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification (Primary and Secondary RFID) reagent card(refer to instrument user manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.

Recalibration is recommended when:

(1) the reagents of different lot are used;

(2) the same lot of reagents were used on the analyzer beyond 28 days;

(3) quality control misses the target.

Handing of lyophilized calibrators and control materials:

Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 10~15 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted calibrators or control materials to the additional bottles. After bottles is marked and dispensed. If you do not test immediately, you need to store the aliquots immediately at -20 ℃ (± 5 ℃).

Quality control

For quality control, use 25-OH VD control materials.

In addition, other suitable control material can be used.

Control materials for the various concentration ranges should be run individually at

least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
If necessary, repeat the measurement of the samples concerned.
Follow the applicable government regulations and local guidelines for quality control.
Please make sure that the correct values are used.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is ng/mL or nmol/L: nmol/L × 0.40 = ng/mL

Specimen dilution

Samples exceeding the measuring range would be manually diluted with 25-OH vitamin D -negative serum.
The recommended dilution ratio is 1:4, the concentration of the diluted sample must be > 30 ng/mL, and the diluted sample test results need to multiply dilution ratio.

【Biological reference interval】

According to serum test results of 240 healthy human (male 120, female 120) , the reference intervial is >30 ng/mL.
Most experts agree that vitamin D deficiency should be defined as 25-OH vitamin D≤ 20 ng/mL (≤ 50 nmol/L). 25-OH vitamin D insufficiency is recognized as 21-29 ng/mL[6]. Similarly, the US National Kidney Foundation considers levels < 30 ng/mL to be insufficient or deficient[7].The preferred level for 25-OH vitamin D by many experts is now recommended to be ≥ 30 ng/mL (≥ 75 nmol/L) [6].
Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

【Result Interpretation】

For explaining the result, the patient's overall clinical manifestation should be referred to, including symptoms, medical history and other relevant data and information.

【Limitations】

Test results are used only for clinical reference and cannot be used as the basis for diagnosis or rule-out of diseases alone.
When the sample containing bilirubin ≤1129 μmol/L ,triglyceride ≤400 mg/dL, total protein ≤10 g/dL, biotin ≤70 ng/mL, or hemoglobin ≤100 mg/dL, or HAMA (human anti-murine antibodies) ≤ 200 ng/mL, interference deviation to the test result lies within ±10%. The test result would not be interfered by rheumatoid factor (800 IU/mL).

【Measuring Range】

The measuring range of the kit is 3~100 ng/mL. For samples with 25-OH vitamin D content below the lower detection limit, the results are reported as < 3 ng/mL, If the 25-OH vitamin D content is above the upper detection limit, the results are reported as >100 ng/mL.

【Analytical Performance】

Lower limit of measurement

The lower detection limit is 3.0 ng/mL (repeatability study, n = 20).

Accuracy

The ratio of measuring national or international reference materials between the measured value and the theoretical value should fall between 0.900 and 1.100.
Or The ratio of measuring correctness control product of standardized traceability between the measured value and the theoretical value should fall between 0.900 and 1.100.

Linearity

The correlation coefficient (Pearson's r) is not less than 0.9900 in the interval of 3~70 ng/mL.

Within-run precision (repeatability)

The coefficient of variation (CV) ≤ 10%.

Between-lot precision

The coefficient of variation (CV) ≤ 15%.

Analytical specificity

Test results of blank samples spiked with analogs below are summarized in the following table.

Analog	Concentration	Cross-reactivity (%)
Vitamin D2	2000 ng/mL	≤1.0
Vitamin D3	2000 ng/mL	≤1.0
25-OH Vitamin D2	40 ng/mL	≥95
25-OH Vitamin D3	40 ng/mL	≥95
Ibandronate	1mg/mL	≤1.0
ALendronate	1mg/mL	≤1.0
Pamidronate	1mg/mL	≤1.0
ALfacalcidol	1mg/mL	≤1.0
Zoledronate	1mg/mL	≤1.0

【Method Comparison】

A comparison of the Lifotronic 25-OH Vitamin D assay (y) with the Roche Elecsys Vitamin D total Method (x) using clinical samples gave the following correlations:
Number of samples measured: 232
Linear regression:
Y=0.9869 x-0.0633
r = 0.9857
The sample concentrations were between approx 4.90 and 67.63 ng/mL.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.
When using the kit, it's necessary to exercise the normal precautions in the laboratory.
All reagents and samples including specimen, calibrators and control materials, should avoid foaming before and during test.
Test results of the kit are for clinical reference only, and clinical evaluation of patients should be combined with their symptoms and signs, medical history, other laboratory examination results and treatment responses.
Due to factors such as methodology or antibody specificity, testing identical samples with reagents from different manufacturers may get different results, and the results from different kits should not compare directly, lest cause the wrong medical explanation,It's suggested that laboratorians should point out the characteristics of used reagent in the test report to the clinician. In serial monitoring, if the reagent type is changed, a continuous parallel test should be performed and comparison of the results to the former should be to determine new baseline value.
This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.
Any serious incident that would be occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established;
The preservative ProClin™ 300 contains 3% 2-methyl-4-isothiazolin-3-one (MIT) and 5-chloro-2-methyl-4-isothiazolin- 3-one (CMIT), will be harmful if inhalation, contact with skin and/or swallow, and may be toxic to aquatic organisms. The abandoned reagents should be dealed with in compliance with local regulations.

【Symbol Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date	IVD	In vitro diagnostic medical device
LOT	Batch code	Σ	Sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limit		This way up
EC REP	Authorized representative in the European Community	CE	Indicates this device is in compliance with Europe Directive.
REF	Catalogue number	P/N	Material code
	Pay attention to the corrosion		Date of manufacture
	Warning	UDI	Unique device identifier

【Reference】

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3. Houghton LA, Vienth R. The case against ergocalciferol (vitamin D_2) as a Vitamin supplement, *Am J Clin Nutr*, 2006;84:694-697.
4. Peterlik M, Boonen S, et al. Vitamin D and calcium insufficiency-related chronic diseases: An emerging world-wide public health problem. *Int.J Environ Res Public Health*, 2009;6:2585-2607.
5. Grant WB. Estimated benefit of increased vitamin D status in reducing the economic burden of disease in western Europe. *Prog Biophys Mol Biol*. 2009;99:104-113.
6. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol.* 2009;19(2):73-78.
7. KDOQI Clinical Practice Guidelines for Bone Metabolism and Disease in Children With Chronic Kidney Disease.

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