ichroma™ Vitamin D

INTENDED USE

ichroma™ Vitamin D is a fluorescence Immunoassay (FIA) for the quantitative determination of total 25(OH)D2/D3 level in human serum/plasma. It is useful as an aid in management and monitoring of regulating the concentration of calcium and phosphate in the bloodstream and promoting the healthy growth and remodeling of bone.

For *in vitro* diagnostic use only.

INTRODUCTION

Vitamin D from the diet or dermal synthesis from sunlight is biologically inactive and is a fat soluble steroid hormone involved in the active intestinal absorption of calcium and in the regulation of its homeostasis. In humans, the most important compounds in this group are vitamin D3 (also known as cholecalciferol) and vitamin D2 (ergocalciferol). [1] In the liver, cholecalciferol (vitamin D3) is converted to calcidiol, 25-hydroxycholecalciferol (abbreviated 25(OH)D3). Ergocalciferol (vitamin D2) is converted in the liver to 25-hydroxyergocalciferol (25(OH)D2). It is widely known that circulating 25(OH)D is the best indicator of vitamin D status.[2,3] 25(OH)D3 is then converted in the kidneys (by the enzyme 25(OH)D-1α-hydroxylase) into 1,25-$(OH)_2$D3, a steroid hormone that is the active form of vitamin D. It can also be converted into 24-hydroxycalcidiol in the kidneys via 24-hydroxylation.[4,5] 1,25-$(OH)_2$D3 circulates as a hormone in the blood, regulating the concentration of calcium and phosphate in the bloodstream and promoting the healthy growth and remodeling of bone. 1,25-$(OH)_2$D3 also affects neuromuscular and immune function.[6] Vitamin D has a significant role in calcium homeostasis and metabolism. Its discovery was due to effort to find the dietary substance lacking in rickets (the childhood form of osteomalacia).[7]

This test can be used to diagnose vitamin D deficiency, and it is indicated in patients with high risk for vitamin D deficiency and when the results of the test would be used as supporting evidence for beginning aggressive therapies.[8] Patients with osteoporosis, chronic kidney disease, malabsorption, obesity, and some other infections may be high risk and thus have greater indication for this test.[9,10]

PRINCIPLE

The test uses a competitive immunodetection method. In this method, the target material in the sample binds to the fluorescence (FL)-labeled detection antibody in detection buffer, to form the complex as sample mixture. This complex is loaded to migrate onto the nitrocellulose matrix, where the covalent couple of 25(OH)D3 and bovine serum albumin (BSA) is immobilized on a test strip, and interferes with the binding of target material and FL-labeled antibody. If the more target material exists in blood, the less detection antibody is accumulated, resulting in the less fluorescence signal.

COMPONENTS

ichroma™ Vitamin D consists of 'Cartridges', 'Detection Buffer Vial', 'Releasing Buffer Vial', 'Sample Mixing Tubes' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has 25(OH)D3-BSA conjugate at the test line, while rabbit IgG at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The releasing buffer contains NaOH and DMSO.
- The detection buffer contains anti 25(OH)D2/3-fluorescence conjugate, anti rabbit IgG-fluorescence conjugate, gelatin as a stabilizer and sodium azide in Tris-HCl buffer as a preservative.
- The releasing buffer and detection buffer are dispensed in a vial. Releasing buffer vial and detection buffer vial are packed in a Styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip, releasing buffer and detection buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A sample mixing tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer, releasing buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ Vitamin D** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer vial, releasing buffer vial, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- As releasing buffer is basic and contain organic solvent, please avoid contact with eyes, skin or clothing.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ Vitamin D** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ Vitamin D** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **EDTA, heparin, sodium citrate** should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The detection buffer dispensed in a vial is stable for 20 months if stored at 2-8 °C.
- The releasing buffer dispensed in a vial is stable for 20 months if stored at 2-8 °C.
- Opened detection buffer and releasing buffer are stable for 12 months at 2-8 °C if kept capped in original container and free from contaminations.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the

test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-47

Components of **ichroma™ Vitamin D**
- Cartridge Box:
  - Cartridges 25
  - Sample Mixing Tubes 25
  - ID Chip 1
  - Instruction For Use 1
- Detection Buffer Vial (3 mL) 1
- Releasing Buffer Vial (2 mL) 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ Vitamin D**. Please contact our sales division for more information.
- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
- **ichroma™ Printer** REF FPRR007
- **i-chamber** REF FPRR009
- **ichroma™ Vitamin D Control** REF CFPO-79
- **Inserting tube block** (It could be displaced with heating block.)

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Vitamin D** is human serum/plasma.
- It is recommended to test the sample within 24 hours after collection.
- Samples may be stored for up to a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples should be frozen at -20 °C.
- Samples stored frozen at -20 °C for 6 months showed no performance difference.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the contents of **ichroma™ Vitamin D**: Sealed Cartridge, Detection Buffer Vial, Releasing Buffer Vial, Sample Mixing Tubes and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the detection buffer & releasing buffer.
- Keep the sealed cartridge (if stored in refrigerator) and the detection buffer tube at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for ichroma™ tests.
- Insert the ID Chip into the ID chip port of the instrument for ichroma™ tests**.**
- Press the 'Select' button on the instrument for ichroma™ tests.
- Turn on the i-Chamber and set the temperature at 35 °C.
- Insert 'Inserting tube block' into the i-Chamber slot at least 10 min before the test.
  (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

1) Put the test cartridge into the i-Chamber slot.
2) Transfer 50 µL of releasing buffer using a transfer pipette to a sample mixing tube.
3) Add 50 µL sample (Human serum/plasma/control) using a transfer pipette to the sample mixing tube containing releasing buffer and mix well by pipetting 10 times.
4) Insert the sample mixing tube into the inserting tube block and leave the tube in the inserting tube block at 35 °C for 5 min.
5) Add 100 µL of detection buffer using a transfer pipette with new tip to the sample mixing tube containing the releasing buffer and sample mixture.
6) Mix well by pipetting 10 times and leave it in the inserting tube block again at 35 °C for 15 min.
7) Take out the half of test cartridge from the i-Chamber, pipette out 75 µL of incubated mixture and load it into the sample well on the test cartridge. Then push the test cartridge into the i-Chamber slot fully.
8) Leave the sample-loaded test cartridge in i-Chamber for 8 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
9) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
10) Press 'Select' button on the instrument for ichroma™ tests to start the scanning process.
11) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
12) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays total 25(OH)D2/D3 concentration of the test sample in terms of ng/mL.
- The cut-off (reference range)

25(OH)D		status
<10 ng/mL	<25 nmol/L	Deficiency
10-30 ng/mL	25-75 nmol/L	Insufficiency
30-100 ng/mL	75-250 nmol/L	Sufficiency

- Working range: 8.0-70 ng/mL
- Conversion factor: 2.5 x ng/mL = nmol/L

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided with **ichroma™ Vitamin D**. **ichroma™ Vitamin D Control** can be used as a calibration as well as quality control test. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 6.50 ng/mL (16.25 nmol/L)
  - Limit of Detection (LoD) 7.40 ng/mL (18.50 nmol/L)
  - Limit of Quantification (LoQ) 7.99 ng/mL (19.98 nmol/L)
- **Analytical specificity**
  - Cross-reactivity
    There was no significant cross-reactivity from these materials with the **ichroma™ Vitamin D** test measurements.

Cross-reactivity material	Standard material conc. (ng/mL)		
	9.59	23.76	64.78
	Bias (%)		
Vitamin D2 (300 ng/ml)	9.37	8.32	2.97
Vitamin D3 (300 ng/ml)	5.98	6.65	-2.30

- Interference
  There was no significant interference from these materials with the **ichroma™ Vitamin D** test measurements.

Interference material	Standard material conc. (ng/mL)		
	9.59	23.76	64.78
	Bias (%)		
EDTA (2 mg/ml)	0.17	5.44	-2.51
Heparin (200 U/ml)	-1.40	-0.93	-6.03
Sodium citrate (38 mg/ml)	-1.87	6.77	-3.61
Urea (2.6 mg/ml)	7.68	-3.74	-3.64
Ascorbic acid (300 μg/ml)	-2.25	4.14	1.09

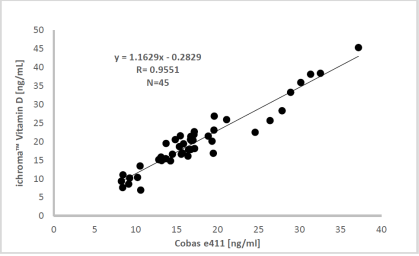
- **Precision**
  - Between lot/person/day/site
    The precision was confirmed by 3 different evaluators with 3 different lots, during 5 days, testing five times each different concentrations.

conc. (ng/mL)	Between-lot		Between-person		Between-day		Between-site	
	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)
9.59	9.63	9.12	9.37	8.80	9.40	11.26	9.53	10.03
23.76	23.08	5.87	23.03	5.87	24.26	6.11	23.02	6.68
64.78	64.29	5.30	64.21	4.65	64.58	4.25	64.64	3.56

- **Accuracy**
  The accuracy was confirmed by 3 different lots testing ten times each different concentrations.

conc. [ng/ml]	Lot1	Lot2	Lot3	AVG	SD	CV (%)	Recovery (%)
9.59	9.56	9.74	9.61	9.63	0.88	9.12	100.46
23.76	22.71	22.84	23.68	23.08	1.35	5.87	97.13
64.78	63.21	65.05	64.62	64.29	3.41	5.30	99.25

- **Comparability:** 25(OH)D concentrations of 45 serum samples were quantified independently with **ichroma™ Vitamin D** and Roche Cobas e411 as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y=1.1629X – 0.2829 and R = 0.9951 respectively.



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Note: Please refer to the table below to identify various symbols

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

For technical assistance; please contact:
Boditech Med Inc.'s Technical Services
Tel: +82 33 243-1400
E-mail: sales@boditech.co.kr

Boditech Med Incorporated
43, Geodudanji 1-gil, Dongnae-myeon,
Chuncheon-si, Gang-won-do, 24398
Republic of Korea
Tel: +(82) -33-243-1400
Fax: +(82) -33-243-9373
www.boditech.co.kr

EC REP **Obelis s.a**
Bd. Général Wahis 53,
1030 Brussels, BELGIUM
Tel: +(32) -2-732-59-54
Fax: +(32) -2-732-60-03
E-Mail: mail@obelis.net

CE