BIO TECHNOLOGY boditech

# 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 sandwich immunodetection method; the detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized-antibodies on test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by instrument for ichroma™ tests to show Vitamin D concentration in the sample.

## COMPONENTS

**ichroma™ Vitamin D** consists of 'cartridges', 'detector tubes', 'releasing buffer', and a 'detector diluent'.

- The cartridge contains the membrane called a test strip, which has streptavidin at the test line, with chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant in a box.
- The detector tube has 2 granules containing vitamin D complex antibody-biotin conjugate, vitamin D capture antibody-fluorescence conjugate and anti-chicken IgY-fluorescence conjugate in phosphate buffered saline. All detector tubes are packed in a pouch.
- The releasing buffer contains sodium azide in DMSO, and it is pre-dispensed in a vial. The releasing buffer is packed in a box.
- The detector diluent contains sodium azide in phosphate buffered saline, and it is pre-dispensed in a vial. The detector diluent is packed in a box.

## WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- It is possible to use frozen samples. Please refer to "SAMPLE COLLECTION AND PROCESSING."
- Lot numbers of all the test components (cartridge, detector tubes, releasing buffer, detector diluent and ID chip) 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 incorrect test result(s).
- Do not reuse cartridges or detector tubes. A cartridge should be used for testing one sample only. A detector tube should be used for processing of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use the cartridge, if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- Allow cartridge, detector tube, releasing buffer, detector diluent, and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detector tubes, releasing buffer, detector diluent, and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- As releasing buffer is basic and contains 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 below conditions.
  - **ichroma™ Vitamin D** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant sample.

| Recommended anticoagulant |
|---|
| $K_2$EDTA, Sodium heparin, Sodium citrate |

## STORAGE AND STABILITY

| Storage condition | | | |
|---|---|---|---|
| Component | Storage Temperature | Shelf life | Note |
| Cartridge | 4 – 30 °C | 20 months | Disposable |
| Detector tube | 4 – 30 °C | 20 months | Disposable |
| Releasing buffer | 4 – 30 °C | 20 months | Unopened |
| | 4 – 30 °C | 3 months | Opened |
| Detector diluent | 4 – 30 °C | 20 months | Unopened |
| | 4 – 30 °C | 3 months | Opened |

- 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(s) due to the non-responsiveness of the antigen to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative result 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:
  - Cartridge 25
  - Detector tube 25
  - Releasing buffer 1
  - Detector diluent 1
  - ID chip 1
  - Instruction for use 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 FP203
  - **ichroma™ II** REF FPRR021
- **Printer** REF FPRR007
- **i-chamber** REF FPRR009
- **Vitamin D Control** REF CFPO-79
- **Inserting tube block** REF CFPO-80

## 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 cartridges, detector tubes, releasing buffer, detector diluent, ID chip and instruction for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, releasing buffer, detector diluent as well as an ID chip.
- If the sealed cartridge, detector tube, releasing buffer and the detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn 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 pipette to a detector tube containing the granules. When the granule form is completely dissolved in the tube, it becomes detection buffer. (The detection buffer must be used within 1 minute.)
3) Add 50 µL sample (Human serum/plasma/control) using a pipette to a detector tube containing releasing buffer and mix well by pipetting 10 times.
4) Insert the detector 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 detector diluent using a pipette with a new tip to the detector 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 inaccurate 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 is marked on the cartridge especially for this purpose.
10) Press the 'Select' or Tap the 'START' button on the instrument for ichroma™ tests to start the scanning process.
11) The 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: 1 ng/mL = 2.5 x 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**. **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 (19.98nmol/L)

- **Analytical specificity**
  - Cross-reactivity
    Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ Vitamin D** test results did not show any significant cross-reactivity with these biomolecules.

| Cross-reactivity material | Standard material conc. (ng/mL) | | |
|---|---|---|---|
| | 7.01 | 21.97 | 65.83 |
| | Bias (%) | | |
| Vitamin D2 (300 ng/ml) | 107.05 | 105.67 | 96.39 |
| Vitamin D3 (300 ng/ml) | 101.83 | 107.14 | 93.29 |

  - Interference
    Interference materials such as below the ones in the table were added to the test sample(s) the same as the below concentrations. **ichroma™ Vitamin D** test results did not show any significant interference with these materials.

| Interference material | Standard material conc. (ng/mL) | | |
|---|---|---|---|
| | 7.01 | 21.97 | 65.83 |
| | Bias (%) | | |
| EDTA (2 mg/ml) | 106.48 | 98.09 | 96.27 |
| Heparin (200 U/ml) | 94.89 | 93.12 | 95.36 |
| Sodium citrate (38 mg/ml) | 94.29 | 93.20 | 95.34 |
| Urea (2.6 mg/ml) | 98.92 | 91.79 | 95.25 |
| Ascorbic acid (300 µg/ml) | 101.70 | 95.64 | 100.63 |

- **Precision**
  - Intra-assay
    One person tested three different lots of **ichroma™ Vitamin D**, ten times at each concentration of the control standard.

| Conc. | Lot 1 | | Lot 2 | | Lot 3 | |
|---|---|---|---|---|---|---|
| (ng/mL) | AVG | CV (%) | AVG | CV (%) | AVG | CV (%) |
| 7.01 | 6.73 | 10.91 | 6.57 | 11.22 | 7.09 | 14.07 |
| 21.97 | 20.53 | 9.06 | 20.67 | 8.03 | 23.31 | 9.76 |
| 65.83 | 64.26 | 3.18 | 63.67 | 2.76 | 65.19 | 2.16 |

  - Inter-assay
    Three different persons tested 3 different lots of **ichroma™ Vitamin D,** three times at each concentration of the control standard.

| Conc. | Between Lot | | Between run | | Total | |
|---|---|---|---|---|---|---|
| (ng/mL) | AVG | CV (%) | AVG | CV (%) | AVG | CV (%) |
| 7.01 | 6.80 | 12.24 | 7.04 | 14.76 | 6.91 | 13.53 |
| 21.97 | 21.51 | 10.63 | 22.63 | 9.74 | 22.04 | 10.43 |
| 65.83 | 64.37 | 4.21 | 63.05 | 5.22 | 63.75 | 4.80 |

■ **Accuracy**

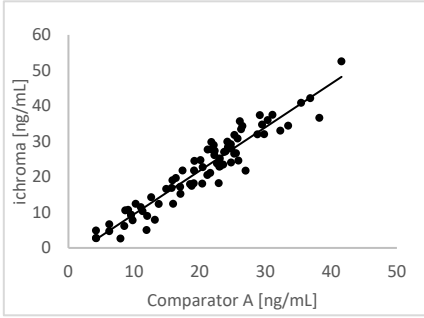
The accuracy was confirmed by 3 different lots testing ten times each different concentrations.

| Conc. | Lot 1 | | Lot 2 | |
|---|---|---|---|---|
| (ng/mL) | AVG | Recovery (%) | AVG | Recovery (%) |
| 7.01 | 7.48 | 106.75 | 7.61 | 108.50 |
| 21.97 | 21.51 | 97.93 | 21.70 | 98.76 |
| 65.83 | 62.71 | 95.27 | 64.00 | 97.22 |

| Conc. | Lot 3 | |
|---|---|---|
| (ng/mL) | AVG | Recovery (%) |
| 7.01 | 7.63 | 108.90 |
| 21.97 | 22.04 | 100.32 |
| 65.83 | 64.51 | 98.00 |

■ **Comparability**

25(OH)D concentrations of 79 serum samples were quantified independently with **ichroma™ Vitamin D** and Comparator A 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 = 1.2267X – 2.7861 and R = 0.9540 respectively.

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

| | |
|---|---|
| | 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 |

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