BIO TECHNOLOGY boditech

Hormone ichroma™

Vitamin D Neo

INTENDED USE

ichroma™ Vitamin D Neo is a fluorescence immunoassay (FIA) for the quantitative determination of Total 25-OH Vitamin D (D2/D3) level in human whole blood/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 seco-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)2D3, a seco-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)2D3 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)2D3 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, migrate onto nitrocellulose matrix to be captured by the other immobilized streptavidin on a test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibody-fluorescence, which is processed by the instrument for ichroma™ tests to show Total 25-OH Vitamin D (D2/D3) concentration in the sample.

COMPONENTS

ichroma™ Vitamin D Neo consists of 'cartridges', 'detector tubes' and 'extraction buffer'.

- The cartridge contains the membrane called a test strip which has streptavidin at the test line and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.
- The detector tube has 2 granules antibody-fluorescence conjugate, antibody-biotin conjugate, anti-chicken IgY-fluorescence conjugate, sodium azide as a preservative in phosphate buffered saline (PBS). All detector tubes are packed in a pouch.
- The extraction buffer contains sodium azide as a preservative in phosphate buffered saline buffer (PBS), and it is pre-dispensed in a vial. The extraction buffer 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.
- Lot numbers of all the test components (cartridge, detector tube, extraction buffer, 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.
- If test components and/or sample are stored in refrigerator, then allow cartridge, detector tube, extraction buffer 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, pipette tips, and capillary tubes should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detector tube and the extraction buffer contain sodium azide (NaN_3), and it may cause certain health issues like convulsions, low blood pressure, low heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in **ichroma™ Vitamin D Neo** when biotin concentration in the sample was below 50 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **ichroma™ Vitamin D Neo** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Vitamin D Neo** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Sodium heparin, Sodium citrate, K_2 EDTA

- **The capillary tube should be used when the following conditions are met.**
  - The capillary tube provided with the kit is recommended to obtain correct test result.
  - Whole blood should be immediately tested after collection.
  - Excess whole blood around the capillary tube should be wiped off.
  - In order to avoid cross-contamination, please do not re-use capillary tube for multiple samples.

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 antigens 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 antigens with time and/or temperature may also cause false negative result as it makes the antigens 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 in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30°C	20 months	Disposable
Detector tube	2 – 30°C	20 months	Disposable
Extraction buffer	2 - 30°C	20 months	Unopened
		3 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

MATERIALS SUPPLIED

REF CFPC-133

Components of **ichroma™ Vitamin D Neo**

- Cartridge box:
  - Cartridge 25
  - Detector tube 25
  - Extraction buffer 1
  - ID chip 1
  - Instructions for Use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ Vitamin D Neo**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M3** REF FPRR035
  - **ichroma™-50 PLUS** REF FPRR036
- **i-Chamber** REF FPRR009
- **Boditech Vitamin D Control** REF CFPO-102
- **30μL Capillary tube** REF CFPT-21

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Vitamin D Neo** is human whole blood/serum/plasma.

- It is recommended to test the sample within 24 hours after collection. When collected sample is stored at room temperature.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (serum, plasma) may be stored for a week at 2 - 8 °C prior to being tested. If testing will be delayed more than a week, samples (serum, plasma) should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.
- Whole blood sample may be used to collect according to below:

  ① Wear disposable gloves and protective equipment for safety.

② Open the zipper bag which has capillary tubes.
③ Take out the capillary tube and check for damage or contamination.
④ Hold the handle of the capillary tube and touch the surface of blood with the capillary tube.
⑤ Fill it with blood completely.
(Make sure that no air bubbles are present in the capillary tube. Do not get blood on the surface of the capillary tube. If the blood gets on the surface of the capillary tube, remove it gently with gauze.)

TEST SETUP

- Check the contents of **ichroma™ Vitamin D Neo**: Sealed cartridges, detector tubes, an extraction buffer, an ID chip and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the extraction buffer as well as an ID chip.
- If the sealed cartridge, the detector tube and the extraction buffer 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 i-Chamber and set temperature at 35 °C.
- Turn on the instrument for ichroma™ tests.

※ **Please refer to the instrument for ichroma™ tests operation manual for complete information and operating instructions.**

CAUTION

- To minimize erroneous test results, we suggest that the ambient temperature of the cartridge should be 35 °C during the reaction time after loading sample mixture to the cartridge.
- To maintain the ambient temperature to 35 °C, you can use various devices such as an i-Chamber or an incubator and so on.

TEST PROCEDURE

▶ **ichroma™ II, ichroma™ M3**

1) Take 150 µL of extraction buffer using a pipette and dispense it to the detector tube containing granules. When the granule form is completely dissolved in the tube, it becomes detection buffer. (The detection buffer must be used immediately. Do not exceed 30 seconds.)
2) Take 30 µL of sample (whole blood, serum/plasma/control) using a pipette and dispense it to the detector tube.
   ※ If 30 µL capillary tube was used to collect the whole blood, put it directly into the detector tube.
3) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately. Do not exceed 30 seconds.)
4) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
5) Insert the sample-loaded cartridge into the slot of the i-Chamber or an incubator (35°C).
6) Leave the sample-loaded cartridge in the i-Chamber or an incubator for 12 minutes.

⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*

7) 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.
8) Tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process. (ichroma™ M3 will start the test automatically after inserting.)
9) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
10) Read the test result on the display screen of the instrument for ichroma™ tests.

■ **ichroma™ III**

1) The test procedure is same with the '1) – 4) of ichroma™ II test procedure'.
2) Insert the sample-loaded cartridge into the 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.
3) Tap the 'Start' button on the instrument for ichroma™ tests.
4) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 12 minutes.
5) Read the test result on the display screen of the instrument for ichroma™ tests.

▶ **ichroma™-50 PLUS**

1) Insert the tip array in the tip station.
2) Insert the detector tube in the reagent station and cover the reagent station to hold the detector tubes in place.
3) Open the lid of the extraction buffer and insert the extraction buffer in the diluent station.
4) Insert the cartridge magazine with the cartridges into the magazine station.
5) Insert the sample tube into the blood collection tube rack and load the blood collection tube rack into the sampling station (loading part).
6) Tap the button located in the upper side of the No. of test cartridge region to select the ID chip that you want to use.
7) When the selected cartridge slot is activated, set the number of the detector tube by tapping.
8) Set the number of pipette tips by tapping.
9) Tap the 'Start' button on the left upper of the main screen to start test.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays Total 25-OH Vitamin D (D2/D3) concentration of the test sample in terms of ng/mL.
- Working range: 5 - 100 ng/mL
- Conversion factor: 1 ng/mL = 2.5 nmol/L
- Reference range

Total 25-OH Vitamin D (D2/D3)		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

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.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with **ichroma™ Vitamin D Neo**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**

Limit of Blank (LoB)	1.61 ng/mL
Limit of Detection (LoD)	2.83 ng/mL
Limit of Quantitation (LoQ)	5.00 ng/mL

- **Analytical specificity**
  - **Cross-reactivity**

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

Cross-reactivity material	Concentration
Vitamin D2	300 ng/mL
Vitamin D3	300 ng/mL

  - **Interference**

Interferents listed in the following table were added to the test sample at the concentration mentioned below. **ichroma™ Vitamin D Neo** test results did not show any significant interference with these materials.

Material	Concentration
D-glucose	600 mM
L-Ascorbic acid	2 mM
Bilirubin[unconjugate]	4 mM
Hemoglobin(human)	20 g/L
Cholesterol	130 mM
triglyceride	100 mg/mL
Biotin	50 ng/mL

- **Precision**
  - Single-site study

Repeatability (within-run precision)
within-laboratory precision (Total precision)
Lot to lot precision
3 Lots of **ichroma™ Vitamin D Neo** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

  - Multi-site study

Reproducibility
1 Lot of **ichroma™ Vitamin D Neo** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Conc. [ng/mL]	Single-site study			
	Repeatability		Within-laboratory precision	
	Mean	CV (%)	Mean	CV (%)
10	9.69	10.94	9.82	10.48
30	30.35	10.38	30.12	10.57
50	50.71	9.86	50.56	9.71
Conc. [ng/mL]	Single-site study		Multi-site study	
	Lot to lot precision		Reproducibility	
	Mean	CV (%)	Mean	CV (%)
10	9.97	10.14	10.04	10.89
30	29.96	10.27	30.62	9.84
50	49.95	9.96	50.65	8.13

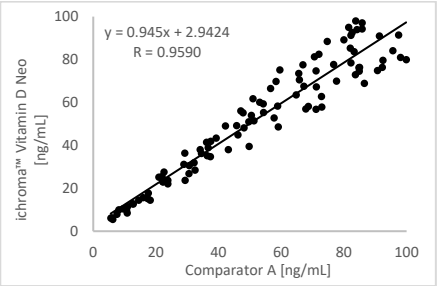
- **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **ichroma™ Vitamin D Neo**. The tests were repeated 10 times at each concentration of the control standard.

Expected value [ng/mL]	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
6.06	6.39	6.23	6.15	6.26	103
10.00	9.97	10.03	10.35	10.12	101
20.83	20.40	21.32	21.34	21.02	101
36.67	36.18	35.26	35.58	35.67	97
52.50	56.33	52.15	53.36	53.95	103
76.25	74.76	82.00	83.48	80.08	105
91.36	88.09	87.80	87.94	87.94	96

- **Comparability**

Vitamin D concentration of 100 clinical samples were quantified independently with **ichroma™ Vitamin D Neo (ichroma™ II)** and **Comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follows.



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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:
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