

FREND™ Vitamin D

Total 25-Hydroxy Vitamin D

Intended use

The FREND™ Vitamin D test is a rapid indirect competitive fluorescent immunoassay designed for the quantitative measurement of 25-Hydroxy vitamin D and related hydroxylated metabolites in human serum and plasma (K_3 EDTA, lithium-heparin and citrate) specimens using the FREND™ System, and the FREND™ AP. Measurements of total 25-hydroxy vitamin D and related hydroxylated metabolites are used to aid in the assessment of vitamin D sufficiency.

The FREND™ Vitamin D microfluidic flow cartridge is designed for use in the FREND™ System fluorescent immunoassay reader, and the FREND™ AP. The FREND™ Vitamin D test system is intended for use in clinical laboratories. For *in vitro* diagnostic use only. The test is not intended for use in point-of-care settings.

Summary and explanation of test

Vitamin D is a fat-soluble prohormone known for its role in regulating calcium and phosphorus levels in bone mineralization.^[1,2] Sunlight exposure produces vitamin D via photochemical conversion of 7-dehydrocholesterol in the epidermis and is the primary source of vitamin D.^[3,4] Seasonal changes, amount of exposure, sunscreen use, and skin pigmentation can cause variation in the amount of vitamin D produced in the body. A minor source of vitamin D can be absorbed from food and vitamin supplements, with an estimated 10-20% absorbed by the body in this manner.^[5,6]

In circulation, 25-OH vitamin D is bound to vitamin D binding protein (VDBP) or albumin at 1000 times higher concentrations than the active form 1,25-(OH)₂-vitamin D. ^[7] Additionally, the 25-OH form has a half-life of 2-3 weeks, as compared to the less stable 1,25-(OH)₂ form, which has a half-life of a few hours. ^[8] 25-OH vitamin D exists as D2 (ergocalciferol) and D3 (cholecalciferol) isomers, with supplements available for both. Often, total 25-OH vitamin D is measured to assess the sufficiency in a patient and make appropriate clinical decisions. ^[9]

Principle of the assay

The FREND™ Vitamin D test cartridge is a single-use rapid “competitive” immunoassay utilizing fluorescent nanoparticles in microfluidic flow to capture and quantify total 25-OH vitamin D in serum and plasma specimens. A 35 µL specimen is placed into a Dilution Tube and 70 µL of the diluted sample is placed into the FREND™ Vitamin D pretreatment tube, which contains proprietary reagents. The pretreatment tube is processed in the FREND™ AP (Advanced Preparing) device. The AP device mixes and incubates the sample and loads the correct volume onto the FREND™ Vitamin D cartridge.

During the incubation labeled 25-OH vitamin D-antibody immune complexes are formed and the AP device transfers a 35 µL sample to the FREND™ Vitamin D cartridge. Vitamin D conjugated fluorescent nanoparticles in the cartridge compete with the 25-OH vitamin D-antibody immune complexes from the pretreatment tube for binding to antibodies immobilized in the test zone. The intensity of fluorescence is inversely proportional to the amount of 25-OH vitamin D in the sample. The FREND™ System measures the fluorescence and calculates, displays, stores and optionally prints the 25- OH Vitamin D concentration.

Material provided

Contents	Catalogue number
20 FREND™ Vitamin D cartridge(s)	FRVDAP 020
20 FREND™ Vitamin D pretreatment tube(s)	
20 FREND™ Vitamin D dilution tube(s)	
40 Disposable pipette tip(s)	
01 FREND™ Vitamin D Code chip	
01 FREND™ Vitamin D Package Insert	

One cartridge contains:

Monoclonal mouse anti-vitamin D
25-hydroxyvitamin D
Fluorescent particles

One dilution tube contains:

Perfluorohexanoic Acid

One pretreatment tube contains

Gold nano-particle conjugation antibody

Materials required but not provided

- The FREND™ System.
- Micro-pipette capable of delivering 35 and 70 µL.
- FREND™ AP.
- Personal protective equipment and biohazard waste.

Warning and Precautions

⚠Caution: *In the United States, federal law restricts this device to sale by or on the order of a physician.*

- The FREND™ Vitamin D cartridges are intended for *in vitro* diagnostic use only.
- Vitamin D cartridges are only to be used on the FREND™ System.
- Vitamin D cartridges are disposable, single use devices. Do not reuse them under any circumstances.
- Allow sealed cartridges to come to room temperature for approximately 15~30 minutes prior to use.
- Cartridges and pretreatment tubes should not be frozen.
- Assure the humidity in the laboratory is in the 10~80% range when tests are run.
- Assure the room temperature remains in the range of 64 °F~77 °F (18~25 °C) when tests are run.
- Avoid cross-contamination between samples by using a new pipette tip for each new specimen.
- Avoid high humidity, direct sunlight or heat in the area used for cartridge storage.
- Inaccurate results are possible if the sample used is contaminated in any way.

- Using specimens containing clotted fibrin could result in erroneous results.
- Over or under loading the cartridge with sample may cause inaccurate results.
- Human specimens are not used in the preparation of this product. However, since human specimens will be used for samples, and since other quality control products in the lab may be derived from human materials please practice Universal Precautions when handling all specimens and controls.
- Do not use the cartridges, pretreatment tubes or dilution tubes beyond the expiration date on the pouch.
- Do not use the cartridge and pretreatment tubes if the pouch is damaged or the seal is broken.
- Perform testing as specified in the Package insert and User manual.
- Keep the cartridge and pretreatment tube sealed in the pouch until ready for use.
- Use the cartridge and pretreatment tube immediately after opening the pouch.
- Wear disposable gloves when handling the cartridges, pretreatment tubes and the samples.
- Wash hands thoroughly and often after handling reagent cartridges or samples.
- Do not ingest the silica gel package found in the cartridge pouch.
- The FREND™ Vitamin D assay has been evaluated and no high dose hook effect was observed for Vitamin D concentrations up to 1600 ng/mL.
- Handle specimens in accordance with the OSHA Standard on Bloodborne Pathogens.^[10]

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature. Reagent stability has been demonstrated for twelve months from the date of manufacture.

The expiration date is clearly indicated on the product box and the cartridges.

Materials

- Refrigerator temperature storage (36~46 °F or 2~8 °C)
FREND™ Vitamin D cartridges, pouched
FREND™ Vitamin D pretreatment tubes, pouched
FREND™ Vitamin D dilution tubes
- Room temperature storage
Pipette tips

Specimen collection and handling

Human serum and plasma (lithium-heparin, K_3 EDTA and citrate) samples are suitable for use with FRENTM Vitamin D cartridges.

Follow instructions detailed in this package insert as well as the specimen collection tube manufacturer's instructions for specimen collection and preparation (including manufacturer's instructions for centrifugation time and speed.)

For serum, a blood sample is collected aseptically without additives by venous puncture. After allowing the sample to clot for 30 minutes at room temperature, the collection tube should be centrifuged for 10 minutes at 3,000 rpm.

For plasma (lithium-heparin, K_3 EDTA and citrate), a venous blood sample is collected aseptically with the designated additive. After allowing the specimen to sufficiently mix with anticoagulant at room temperature, centrifuge the tube for 10 minutes at 3,000 rpm.

Samples may be stored at 36–46 °F (2–8 °C) for up to 6 hours prior to analysis. If the analysis is scheduled to be done at some later time, the serum or plasma sample should be separated from the red cells and stored frozen at –4 °F (–20°C) or below for future use.

Repeated freeze-thaw cycles should be avoided. Prior to assay, slowly bring frozen samples to room temperature (64–77 °F or 18–25 °C) and mix gently but thoroughly before testing.

For optimal results, avoid grossly hemolytic, lipemic, or turbid specimens. Specimens should be free of aggregated fibrin, red blood cells, or other particulate matter.

Procedure

Calibration

There is no need for calibration to be performed by the end user as is generally required on other automated laboratory equipment. All calibration statistics and information have been electronically stored on the FRENDS™ Vitamin D Code chip included in each box of FRENDS™ Vitamin D cartridges. The FRENDS™ Vitamin D Code chip is specific for each lot of FRENDS™ Vitamin D cartridges.

Calibration information should always be checked by running external quality control samples to verify that the results obtained for Vitamin D on the FRENDS™ System using the FRENDS™ Vitamin D cartridges meet the laboratory criteria for acceptability.

Code chip installation

Please refer to the FRENDS™ System User manual for more detailed instructions relative to the Code chip installation. Abbreviated instructions follow here:

- 1) Insert the FRENDS™ System electrical cord into an appropriate outlet.
- 2) Insert the Code chip into the Code chip slot at the rear of the FRENDS™ System following the arrows.
- 3) Press the **'Setup'** button on the **'Main'** screen.
- 4) Press the **'Code chip'** button on the **'Setup'** screen.
- 5) The information embedded on the FRENDS™ Vitamin D Code chip is automatically saved on the FRENDS™ System.
- 6) When the Code chip installation is completed, press the **'OK'** button to go to the **'Setup'** screen.
- 7) Press the **'Item'** button on the **'Setup'** screen.
- 8) Check the FRENDS™ Vitamin D cartridge lot number and the installation date of the Code chip.
- 9) Press the **'Home'** button to go to the **'Main'** screen to begin running external quality control or patient samples.

Quality control

• FRENDS™ System QC cartridges

The FRENDS™ QC Cartridge contains multiple controls that check the optics of the system. By testing the QC Cartridge, the integrity of the laser power, alignment and mechanical components of the system are confirmed.

For each day of patient testing perform QC Cartridge testing. Refer to the quality control procedures section in the User Manual of the FRENDS™ System. In brief, perform QC Cartridge testing for the following conditions:

- (1) Upon initial setup of the system,
- (2) Each day of patient testing,
- (3) When the system has been transported or moved,
- (4) Whenever there is uncertainty about the performance of the system,
- (5) Whenever required by your laboratory's quality control requirements.

• Internal procedural controls

The FRENDS™ Vitamin D test cartridge contains a built-in control feature. Fluorescence signal in the reference zone of each cartridge shows: (1) that enough volume is added, (2) that proper flow is obtained, and (3) that the antibody is reactive. If this reference zone signal is missing or lower than threshold, the FRENDS™ System considers it an incorrect or failed test and produces an error message instead of a test result. In addition, with each cartridge run, the system monitors for (1) flow of sample, (2) speed of sample flow, (3) shelf-life of cartridge components, (4) function of internal barcode scanner, and (5) function of scanner's mechanical components.

• External quality control testing

Commercially available controls from a variety of manufacturers are available that contain 25-OH Vitamin D as a measured analyte. It is recommended that a minimum of two (2) levels of controls be run once per day on days when assaying patient samples on the FRENDS™ Vitamin D Test. Or another QC option may be adopted – Individualized Quality Control Plan (IQCP)^[11] is an all-inclusive approach to assuring the quality of the entire testing process. An IQCP includes practices, data and information that each laboratory already uses to ensure quality testing and meet CLIA, beyond testing a certain number of QC materials at a designated frequency.

To ensure that these control procedures are equivalent to CLIA QC regulations and suitable for each laboratory, it is required to establish and document the QC that is appropriate for the test system, testing environment and testing personnel.

Controls should be run according to local, state, federal regulations or accreditation requirements for quality control frequency. If any external quality control sample values are out of the acceptable range, it will be necessary to investigate the problem before reporting patient results to assure there is not an instrument or software malfunction. Do not assay patient samples on the FRENDS™ System using FRENDS™ Vitamin D if quality control results do not fall within the acceptable ranges.

Specimen processing

• Preparation

Remove sufficient FRENDS™ Vitamin D cartridges, Sample dilution tubes, and Gold Antibody pretreatment tubes from the refrigerator to test the number of patient samples and required external quality control materials. Allow the tubes and the sealed pouches containing the cartridges to come to room temperature for approximately 15~30 minutes prior to the start of the testing sequence.

When using refrigerated patient samples, remove those from the refrigerator and allow them to come to room temperature prior to testing. If frozen samples will be utilized, be sure these are removed from the freezer, thawed at room temperature and then mixed gently but thoroughly prior to testing. Testing should not begin on previously frozen samples until they have reached room temperature (64~77 °F or 18~25 °C).

There are no other reagents or sample preparations necessary.

• Assay procedure

- 1) Ensure that the FRENDS™ Vitamin D cartridge, pretreatment tube, Sample Dilution Tube and specimen are at room temperature (64~77 °F or 18~25 °C). Open the pouch and place the FRENDS™ Vitamin D cartridge into the cartridge tray of the AP device. Press “**NEXT**” to close the cartridge tray and open the pretreatment tube tray.
- 2) Transfer 35µL of specimen into the Sample Dilution tube and mix well by vortexing or inverting.

- 3) Transfer a 70 μL aliquot of the diluted specimen to the FRENDD™ Vitamin D pretreatment tube.

⚠ Caution: *Once the sample is added to the pretreatment tube Do not invert the tube.*

- 4) Insert the pretreatment tube into the tube hole in the FRENDD™ AP pretreatment tube tray. Refer to the FRENDD™ AP User manual for complete operating instructions.
- 5) Press the “**NEXT**” button. The pretreatment tray will close and the first incubation step (15 minutes) will begin.
- 6) After the first incubation is complete, 35 μL of mixed sample will be loaded onto the cartridge and the second incubation step (2 minutes) will begin.
- 7) When both incubation steps are completed, the cartridge tray will open and the cartridge will be ready to be inserted into the FRENDD™ System. Press the ‘**Test**’ button on the ‘**Main**’ screen of the FRENDD™ System.
- 8) The system moves to the Patient ID screen automatically.
- 9) Type the Patient ID and press the ‘**Enter**’ button to begin the test.
- 10) Insert the cartridge into the cartridge slot using the cartridge arrow as a guide.

⚠ Caution: *Check the direction of the cartridge before insertion and assure the insertion is complete.*

- 11) When the reaction in the cartridge is completed, the FRENDD™ System will automatically begin the reading process.
- 12) When the measurements are completed, the cartridge will automatically be expelled and the results displayed.

⚠ Caution: *Do not disconnect power cord or shut off power on the FRENDD™ System while a cartridge is in the reading chamber. This may cause a system error.*

- 13) If the FRENDD™ System is connected to the optional printer, press the ‘**Print**’ button and the results will be output on the printer paper.
- 14) For more detailed instructions, please refer to the FRENDD™ System User manual.




Procedural notes

If the Vitamin D concentration of a specimen is found to be greater than the linearity limit of the assay of 110.00 ng/mL, report any value above 110.00 ng/mL as “greater than 110.00 ng/mL”. There is no specific diluent provided in the FREND™ Vitamin D cartridge.

Calculation of results

The FREND™ System performs all sample and reagent handling operations automatically within the cartridge once the sample has been loaded into the sample inlet in the cartridge and the cartridge placed into the FREND™ System. The rate of fluorescence produced by the reaction is read at various intervals during the analysis process, blank readings are subtracted after which the net rate is automatically converted to Vitamin D concentration in ng/mL based upon information stored on the FREND™ Vitamin D Code chip. This result is then output on the screen and to the optional printer. It is also stored in memory on the FREND™ System.

Screen displayed for various concentration scenarios

Displayed result	Description
	Vitamin D concentration Less than 10.00 ng/mL
	Vitamin D concentration Not less than 10.00 ng/mL and not higher than 110.00 ng/mL
	Vitamin D concentration Higher than 110.00 ng/mL

Limitations of the procedure

- 1) When used for diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g., symptoms, results of other tests, clinical impressions, medical history, therapy, etc.)
- 2) The FRENDS™ System paired with a FRENDS™ Vitamin D cartridge, is programmed to report 110.00 ng/mL as the highest concentration of Vitamin D measurable. The lowest measurable concentration is 13.0 ng/mL, the assay limit of detection.
- 3) Specimens from patients with heterophilic antibodies, such as anti-mouse (HAMA), anti-goat (HAGA), or anti-rabbit (HARA) antibodies, may show falsely elevated or depressed values or may result in an incomplete test.^[12, 13] Patients routinely exposed to animals or animal serum products can be prone to these types of heterophilic interferences.
- 4) Patients taking the drugs containing Paricalcitol (e.g., Zemplar) should not be tested by this assay.
- 5) Certain medications may interfere with assay performance. All results should be interpreted with respect to the clinical picture of the patient.
- 6) Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.
- 7) Lipemia has an insignificant effect on the assay except in the case of gross lipemia where interference with the lateral flow of the sample in the cartridge may occur.
- 8) The concentration of Vitamin D in a given sample determined with assays from different manufacturers can vary due to differences in assay methods, calibration, and antibody specificity.
- 9) Please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this Package insert.
- 10) FRENDS™ Vitamin D has not been evaluated in point-of-care settings.
- 11) FRENDS™ Vitamin D is to be used in licensed clinical laboratories with trained technologists.

Performance characteristics

Performance characteristics were evaluated for the FREND™ Vitamin D as follows:

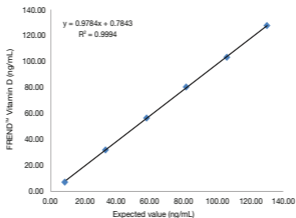
Precision

A precision study for FREND™ Vitamin D was performed as outlined in CLSI guideline EP5-A3. Five human serum based samples were assayed in duplicates, twice per day over a period of 20 days.

sample ID	Mean Conc. (ng/mL)	Repeatability		Between-run		Between-day		Within-laboratory	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	18.50	1.443	8.0	0.348	1.9	0.282	1.6	1.511	8.4
2	28.83	1.643	5.5	0.655	2.2	0.166	0.6	1.777	5.9
3	61.13	3.112	5.1	0.893	1.5	1.142	1.9	3.433	5.7
4	90.04	4.853	5.4	2.201	2.5	0.787	0.9	5.386	6.0
5	119.16	5.486	4.6	2.606	2.2	1.333	1.1	6.218	5.2

Dilution linearity

The dilution linearity study as outlined in CLSI guideline EP6-A was performed by diluting a high concentration Vitamin D specimen with a low concentration Vitamin D specimen. Linearity was demonstrated from 8.00 ng/mL to 130.00 ng/mL.



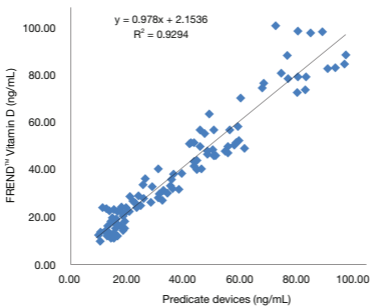
Analytical sensitivity

The limit of blank (LoB) and limit of detection (LoD) were determined according to CLSI guideline EP17-A2. LoB was determined by 60 replicates of a vitamin D depleted serum specimens. LoD was determined by 12 replicates of five low level patient specimens.

LoB (ng/mL)	LoD (ng/mL)	LoQ (ng/mL)
3.84	7.07	7.07

Method comparison

FREND™ Vitamin D was compared to the predicate device as outlined in CLSI guideline EP9-A3. Specimens (n=120) were measured in duplicate on both systems. Linear regression analysis demonstrated a correlation coefficient (R^2) of ≥ 0.92 .



Specificity

The following substances were evaluated for potential cross-reactivity with the FREND™ Vitamin D at three concentrations. Testing was done according to the CLSI guideline EP7-A2. No significant cross-reactivity was found.

Substance	Concentration	Vitamin D concentration level		
		Low	Median	High
		Cross reactivity (%)		
Vitamin D2	500 ng/mL	0.0	0.8	0.8
Vitamin D3	500 ng/mL	0.2	-0.8	-0.5
1,25-(OH) ₂ -Vitamin D2	100 ng/mL	-0.7	-1.0	2.2
1,25-(OH) ₂ -Vitamin D3	100 ng/mL	0.0	0.9	0.3
3-epi-25(OH) Vitamin D3	400 ng/mL	-0.8	-1.2	-1.8
25(OH) Vitamin D2	25 ng/mL	103.2	106.5	96.7
25(OH) Vitamin D3	25 ng/mL	113.5	99.4	97.7

Interference















The interference study was performed as recommended in the CLSI guideline EP7-A2 using three concentrations of Vitamin D. Recovery within 90% to 110% of the expected Vitamin D was considered as lack of interference. No interference by the substances below was found.

Substance	Concentration
Bilirubin	2.5 mg/dL
Biotin	30.0 mg/mL
Triglyceride	370.0 mg/dL
Human albumin	10.7 g/dL
Hemoglobin	200.0 mg/dL

References

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Glossary of symbols

	Caution, warning, Consult accompanying documents
	Catalogue number/Reference number
	Lot number/Batch number
	Use by YYYY-MM-DD or YYYY-MM
	Manufacturer
	Authorized representative in the European Community
	CE marking
	<i>In vitro</i> diagnostic medical device
	Temperature limitation
	Contains sufficient for <n> tests
	Do not reuse
	Do not use if package is damaged
	For prescription use only
	Irritant



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