

## Accurate, traceable 25-Hydroxy Vitamin D testing on a random-access analyzer

Fully automated, highly specific Total Vitamin D results, with a wide measurement range

Although called a vitamin, Vitamin D is a prohormone<sup>1</sup> that is of increasing importance in medical care. Vitamin D promotes calcium absorption<sup>2</sup>, as well as reducing inflammation and modulating cell growth and neuromuscular and immune function<sup>3</sup>. Many genes regulating cell proliferation and differentiation are modulated in part by Vitamin D<sup>4</sup>. There is a significant amount of data connecting Vitamin D deficiency to a wide variety of serious health risks<sup>5</sup> which include osteoporosis<sup>6</sup>, hypertension<sup>7</sup>, cancer<sup>8</sup>, and other diseases<sup>9</sup>.

Dietary intake is only a minor source of Vitamin D; the largest source is the action of sunlight on skin. There are two (2) biologically relevant forms which are known as Vitamin D<sub>2</sub> (ergocalciferol) and Vitamin D<sub>3</sub> (cholecalciferol). Cholecalciferol is the biologically active form<sup>10</sup>. The Thermo Scientific™ Cascadion™ SM Vitamin D assay measures both Vitamin D<sub>2</sub> and Vitamin D<sub>3</sub> and reports the sum as Total Vitamin D.

Multiple studies have shown that up to 90% of the global population, depending on location and season, suffers from Vitamin D deficiency or insufficiency<sup>11, 12</sup>, making supplementation necessary.

Complicating the demand for testing is the lack of consistency in test results. Vitamin D assays are subject to interference from many sources, from Vitamin D metabolites, to common dietary supplements<sup>13</sup>. This interference may mask insufficiency and delay necessary treatment.

The Centers for Disease Control and Prevention (CDC) has established the liquid chromatography – tandem mass spectroscopy (LC-MS/MS) method as the reference method for Vitamin D testing. This method eliminates the interferences and measures only the relevant fractions ergocalciferol (D<sub>2</sub>) and cholecalciferol (D<sub>3</sub>)<sup>14,15</sup>. The drawback of LC-MS/MS is the technical difficulty and hands-on time requirements.

To enable standardization of 25-Hydroxy Vitamin D measurements, three accepted reference method procedures and associated standardization programs (Ghent University, CDC, and NIST) have been established<sup>16</sup>. The performance criteria required by these programs call for a mean bias of ≤5% and an overall imprecision of <10%, across a measurement range of 22.5 – 275 ng/mL<sup>17</sup>. Thermo Fisher Scientific is now an active participant in the Vitamin D Standardization and Certification Program (VDSCP).



**The Cascadion SM 25-Hydroxy Vitamin D assay is the first LC-MS/MS 25-Hydroxy Vitamin D assay to be run on an easy to use fully automated clinical analyzer. The assay maximizes accuracy by measuring 100% of both the 25-Hydroxy Vitamin D<sub>2</sub> and D<sub>3</sub> fractions and excluding the C3 epimers. Total Vitamin D is reported, with the D<sub>2</sub> and D<sub>3</sub> results available to the user and recorded in the instrument software. Calibrators are traceable to the NIST standards, ensuring accurate results.**

## General Information

Total 25-Hydroxy Vitamin D is obtained by first measuring the 25-Hydroxy Vitamin D<sub>2</sub> and 25-Hydroxy D<sub>3</sub> results and then adding those to calculate the Total 25-Hydroxy Vitamin D result.

Compound	LOQ	Analytic Measuring Range
25-Hydroxy Vitamin D <sub>2</sub>	3.4 ng/mL (8.24 nmol/L)	3.4 ng/mL – 132 ng/mL (8.24 – 320 nmol/L)
25-Hydroxy Vitamin D <sub>3</sub>	3.4 ng/mL (8.49 nmol/L)	3.4 ng/mL – 132 ng/mL (8.49 – 329 nmol/L)
Total 25-Hydroxy Vitamin D	3.4 ng/mL	3.4 ng/mL – 264 ng/mL



## Specificity

The specificity of the Vitamin D assay was tested by spiking donor serum or plasma samples with substances of similar chemical structure, known interfering substances, and other endogenous and exogenous substances. Testing was carried out according to the Clinical Laboratory Standards Institute (CLSI) guidelines<sup>18</sup>. Tested substances showing a <10% bias were designated as non-interfering.

**Table A. Selected Endogenous and Exogenous Compounds**

Compound	Test Concentration	Compound	Test Concentration
Bilirubin, Conjugated and Unconjugated	40 mg/dL	Biotin	3500 ng/mL
Cholesterol	500 mg/dL	Ascorbic Acid	5 mg/mL
Triglycerides	2000 mg/dL	Acetaminophen	0.2 mg/mL
β-D-Glucose	10 mg/mL	Pantoprazole	30.0 µg/mL
Hemoglobin	1000 mg/dL	Loratadine	87.0 ng/mL
Rheumatoid Factor	400 IU/mL	Human γ Globulin, Human Albumin	6 g/dL

**Table B. Selected Metabolites and Compounds with Similar Chemical Structures**

Compound	Test Concentration	Compound	Test Concentration
1,25-(OH) <sub>2</sub> Vitamin D <sub>2</sub>	10 ng/mL	3-Epi-1,25-(OH) <sub>2</sub> Vitamin D <sub>2</sub>	10 ng/mL
1,25-(OH) <sub>2</sub> Vitamin D <sub>3</sub>	10 ng/mL	3-Epi-1,25-(OH) <sub>2</sub> Vitamin D <sub>3</sub>	10 ng/mL
Vitamin D <sub>2</sub> (Ergocalciferol)	750 ng/mL	3-Epi-25-(OH) Vitamin D <sub>2</sub>	72.5 ng/mL
Vitamin D <sub>3</sub> (Cholecalciferol)	750 ng/mL	3-Epi-25-(OH) Vitamin D <sub>3</sub>	100 ng/mL
25(OH) Vitamin D <sub>2</sub>	300 ng/mL	25,26-(OH) <sub>2</sub> Vitamin D <sub>3</sub>	10 ng/mL
25(OH) Vitamin D <sub>3</sub>	300 ng/mL	Cholic Acid	12 µg/mL
24,25 (OH) <sub>2</sub> Vitamin D <sub>2</sub>	150 ng/mL	Sitagliptin	1.15 µg/mL
24,25 (OH) <sub>2</sub> Vitamin D <sub>3</sub>	150 ng/mL	Mesoridazine	3.15 µg/mL

## Assay Precision

Precision was evaluated according to CLSI standard EP05-A3 by EP Evaluator software<sup>19</sup>. Serum samples were spiked or diluted to provide six (6) concentrations of Vitamin D<sub>2</sub> and Vitamin D<sub>3</sub> over the range of <3.7 ng/mL to 109 ng/mL. Two replicates of each level were run twice daily for 7 days on each of three (3) Cascadion analyzers. The Cascadion SM Clinical Analyzer calculates Total Vitamin D as the sum of D<sub>2</sub> and D<sub>3</sub>.

Analyte	Test Sample	N	Mean (ng/mL)	Repeatability		Between-run		Between-day		Within-Laboratory	
				SD	CV%	SD	CV%	SD	CV%	SD	CV%
25-Hydroxy Vitamin D <sub>2</sub>	1	84	No measurable 25-Hydroxy Vitamin D <sub>2</sub> concentration								
	6	84	No measurable 25-Hydroxy Vitamin D <sub>2</sub> concentration								
	4	84	5.24	0.301	5.7	0.056	1.1	0.093	1.8	0.32	6.1
	2	84	26.15	0.768	2.9	0.000	0.0	0.463	1.8	0.897	3.4
	3	84	8.57	0.362	4.2	0.000	0.0	0.146	1.7	0.391	4.6
	5	84	104.54	2.562	2.5	2.101	2.0	0.000	0.0	3.313	3.2
25-Hydroxy Vitamin D <sub>3</sub>	1	84	5.95	0.434	7.3	0.028	0.5	0.131	2.2	0.454	7.6
	6	84	14.13	0.597	4.2	0.155	1.1	0.282	2.0	0.678	4.8
	4	84	24.08	0.689	2.9	0.127	0.5	0.000	0.0	0.701	2.9
	2	84	29.94	0.813	2.7	0.000	0.0	0.491	1.6	0.950	3.2
	3	84	102.94	2.615	2.5	0.992	1.0	1.982	1.9	3.428	3.3
	5	84	10.07	0.507	5.0	0.000	0.0	0.239	2.4	0.561	5.6
Total 25-Hydroxy Vitamin D	1	84	5.95	0.434	7.3	0.028	0.5	0.131	2.2	0.454	7.6
	6	84	14.13	0.597	4.2	0.155	1.1	0.282	2.0	0.678	4.8
	4	84	29.32	0.766	2.6	0.000	0.0	0.000	0.0	0.766	2.6
	2	84	56.09	1.130	2.0	0.000	0.0	0.668	1.2	1.313	2.3
	3	84	111.51	2.676	2.4	0.825	0.7	2.057	1.8	3.474	3.1
	5	84	114.61	2.600	2.3	2.275	2.0	0.000	0.0	3.455	3.0



### Key Product Features

Key Product Features	
Sample Material	Human Serum and Plasma
Validated sample collection tubes	16 tubes
Calibration Frequency	30 days
Internal Standard Stability on-board	30 days

- Excludes C3 Epimers, measuring only D<sub>2</sub> and D<sub>3</sub>, with Total 25-OH-Vitamin D reported and the D<sub>2</sub> and D<sub>3</sub> results available to the user
- Ready to use Internal Standard, Calibrators, and Controls
- NIST-traceable Calibrators and Controls

## Ordering Information

Item	Part Number	Item	Part Number
Cascadion SM 25-Hydroxy Vitamin D Calibrator Set	10018760	Probe Wash Solution 1	T001252500
Cascadion SM 25-Hydroxy Vitamin D Control 1	10018761	Probe Wash Solution 2	T001262500
Cascadion SM 25-Hydroxy Vitamin D Control 2	10018762	Probe Wash Solution 3	MB124-212
Cascadion SM 25-Hydroxy Vitamin D Control 3	10018763	Cascadion Evaporation Caps	991100
Cascadion SM 25-Hydroxy Vitamin D Internal Standard	10018764	Quick Connect Cartridge C	CH-955002
Solvent A	MB123-1	Cascadion Mass Focus Solution	990910
Solvent B	MB122-1	Sample Cup 0.5 mL 1000 PCS	989220
Solvent C	MB124-1		

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