

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 prohormone¹ that is of increasing importance in medical care. Vitamin D promotes calcium absorption², as well as reducing inflammation and modulating cell growth and neuromuscular and immune function³. Many genes regulating cell proliferation and differentiation are modulated in part by Vitamin D⁴. There is a significant amount of data connecting Vitamin D deficiency to a wide variety of serious health risks⁵ which include osteoporosis⁶, hypertension⁷, cancer⁸, and other diseases⁹.

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 D₂ (ergocalciferol) and Vitamin D₃ (cholecalciferol). Cholecalciferol is the biologically active form¹⁰. The Thermo Scientific™ Cascadion™ SM Vitamin D assay measures both Vitamin D₂ and Vitamin D₃ 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^{11, 12}, 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 supplements¹³. 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 (D₂) and cholecalciferol (D₃)^{14,15}. 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 established¹⁶. 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/mL¹⁷. 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 D₂ and D₃ fractions and excluding the C3 epimers. Total Vitamin D is reported, with the D₂ and D₃ results available to the user and recorded in the instrument software. Calibrators are traceable to the NIST standards, ensuring accurate results.

Specificity

The specificity of the Vitamin D assay was tested by spiking donor serum 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) guidelines¹⁸. 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) ₂ Vitamin D ₂ | 10 ng/mL | 3-Epi-1,25-(OH) ₂ Vitamin D ₂ | 10 ng/mL |
| 1,25-(OH) ₂ Vitamin D ₃ | 10 ng/mL | 3-Epi-1,25-(OH) ₂ Vitamin D ₃ | 10 ng/mL |
| Vitamin D ₂ (Ergocalciferol) | 750 ng/mL | 3-Epi-25-(OH) Vitamin D ₂ | 72.5 ng/mL |
| Vitamin D ₃ (Cholecalciferol) | 750 ng/mL | 3-Epi-25-(OH) Vitamin D ₃ | 100 ng/mL |
| 25(OH) Vitamin D ₂ | 300 ng/mL | 25,26-(OH) ₂ Vitamin D ₃ | 10 ng/mL |
| 25(OH) Vitamin D ₃ | 300 ng/mL | Cholic Acid | 12 µg/mL |
| 24,25 (OH) ₂ Vitamin D ₂ | 150 ng/mL | Sitagliptin | 1.15 µg/mL |
| 24,25 (OH) ₂ Vitamin D ₃ | 150 ng/mL | Mesoridazine | 3.15 µg/mL |

Assay Precision

Precision was evaluated according to CLSI standard EP05-A3 by EP Evaluator software¹⁹. Serum samples were spiked or diluted to provide six (6) concentrations of Vitamin D₂ and Vitamin D₃ 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 D₂ and D₃.

| Sample | N | Vitamin D ₂ | | | Vitamin D ₃ | | | Total Vitamin D (D ₂ + D ₃) | | |
|--------|----|--|-------|-----------|------------------------|-------|-----------|--|-------|-----------|
| | | Mean Value | SD | Total %CV | Mean Value | SD | Total %CV | Mean Value | SD | Total %CV |
| 1 | 84 | No measurable D ₂ concentration | | | 5.95 | 0.454 | 7.6 | 5.95 | 0.454 | 7.6 |
| 6 | 84 | No measurable D ₂ concentration | | | 14.13 | 0.678 | 4.8 | 14.13 | 0.678 | 4.8 |
| 4 | 84 | 5.24 | 0.320 | 6.1 | 24.08 | 0.701 | 2.9 | 29.32 | 0.766 | 2.6 |
| 2 | 84 | 26.15 | 0.897 | 3.4 | 29.94 | 0.950 | 3.2 | 56.09 | 1.313 | 2.3 |
| 3 | 84 | 8.57 | 0.391 | 4.6 | 102.94 | 3.428 | 3.3 | 111.51 | 3.474 | 3.1 |
| 5 | 84 | 104.54 | 3.313 | 3.2 | 10.07 | 0.561 | 5.6 | 114.61 | 3.455 | 3.0 |

General Information

Total 25-Hydroxy Vitamin D is obtained by first measuring the 25-Hydroxy Vitamin D₂ and 25-Hydroxy D₃ results and then adding those to calculate the Total 25-Hydroxy Vitamin D result.

| Compound | LOQ | Analytic Measuring Range |
|-----------------------------------|-----------|--------------------------|
| 25-Hydroxy Vitamin D ₂ | 3.4 ng/mL | 3.4 ng/mL – 132 ng/mL |
| 25-Hydroxy Vitamin D ₃ | 3.4 ng/mL | 3.4 ng/mL – 132 ng/mL |
| Total 25-Hydroxy Vitamin D | 3.4 ng/mL | 3.4 ng/mL – 264 ng/mL |

| Key Product Features | |
|--------------------------------------|-------------|
| Sample Material | Human Serum |
| Validated sample collection tubes | 8 tubes |
| Calibration Frequency | 30 days |
| Internal Standard Stability on-board | 30 days |

- Excludes C3 Epimers, measuring only D₂ and D₃, with Total 25-OH-Vitamin D reported and the D₂ and D₃ 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 | 10026927 | Probe Wash Solution 1 | T001252500 |
| Cascadion SM 25-Hydroxy Vitamin D Control 1 | 10026928 | Probe Wash Solution 2 | T001262500 |
| Cascadion SM 25-Hydroxy Vitamin D Control 2 | 10026929 | Probe Wash Solution 3 | MB124-212 |
| Cascadion SM 25-Hydroxy Vitamin D Control 3 | 10026930 | Cascadion Evaporation Caps | 991100 |
| Cascadion SM 25-Hydroxy Vitamin D Internal Standard | 10026958 | Quick Connect Cartridge C | CH-955002 |
| Solvent A | MB123-1 | Cascadion Mass Tuning Solution | 990900 |
| Solvent B | MB122-1 | Sample Cup 0.5 mL 1000 PCS | 989220 |
| Solvent C | MB124-1 | Sample Cup 2 mL 1000 PCS | 989221 |

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