Biocrates® Vitamin D Kit: Standardized and Quality-Controlled Vitamin D Testing

Mass Spectrometry Based Accurate Assessment of Vitamin D Status
Clinical Background

The endogenous fraction of vitamin D is synthesised in the human skin from endogenous cholesterol by exposition to UV-light or obtained dietarily. The biologically active form, 1α, 25-dihydroxyvitamin D (calcitriol), is mainly produced in the liver and in the kidney.

Vitamin D is essential for the calcium and phosphate metabolisms, by its regulatory function. By increasing bone mineralisation, vitamin D is a key factor for bone and teeth formation. Therefore, vitamin D is a prevention factor against osteoporosis. Furthermore, vitamin D increases the reabsorption of calcium in the kidneys.

It is well-known that vitamin D-binding receptors are found in several organs and tissues of the human body. Through these receptors vitamin D regulates and influences a big number of biological activities and processes such as modulation of immune-cells or the differentiation of epithel cells. Positive effects were also verified for osteomalacia, chronic renal insufficiency and for the prevention of rickets in children.

The supply situation is determined by the quantitation of total 25-hydroxyvitamin D in blood, because it is the storage form of vitamin D in human beings. Total 25-hydroxyvitamin D is the sum of the two metabolites 25-hydroxyvitamin D2 (uptaken by diet) und 25-hydroxyvitamin D3 (produced endogenous by UV-light).

There is not a unique guideline about a healthy total 25-hydroxyvitamin D level, but generally values less than 10 ng/ml are defined as deficiency, 10-19.9 ng/ml as insufficiency, 20-29.9 ng/ml as hypovitaminosis, 30-149 ng/ml as optimal and values above 150 ng/ml as intoxication.

Nowadays the quantitative analysis of total 25-hydroxyvitamin D is performed by two techniques: immunoassay (IA) and LC-MS/MS.

Advantages of LC-MS/MS Testing

The inability to differentiate between 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 is the main drawback of immune-based techniques. The separate measurements are useful for both, clinical and research purposes.

Moreover, the cross reactivity with other non targeted vitamin D metabolites can only be accounted statistically. It was found that an IA overestimates 25-hydroxyvitamin D3 and underestimates 25-hydroxyvitamin D2 concentrations.

For these reasons, LC-MS/MS based methods for the quantitation of total 25-hydroxyvitamin D replaced IAs in many laboratories and became the “golden standard”.

**BIOCRATES Vitamin D Testing solutions (Bioresat® Vitamin D Kit, Bioresat® Vitamin D Reagents) are all traceable to the new NIST SRM 972a standard hence enhancing interlaboratory harmonization.**

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**Vitamin D – Advantages of LC-MS/MS**

DGKL ring trial for 25-hydroxyvitamin D3:

- Switch to LC-MS reference data as true values
- LC-MS definitely established as “golden standard” in the community
- IA: higher bias and clear underestimation of 25-hydroxyvitamin D3

**Source:** Referenzinstitut für Bioanalytik (DGKL). Report of the DGKL ring trial for vitamins. (31. 08. 2013)
**Biocrates® Vitamin D Kit – Fast and Reliable Vitamin D-Determination**

The **Biocrates Vitamin D Kit** provides ready-to-use components for an LC-MS/MS-based assay and enables the quantitative measurement of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in human serum.

- **Ready to use LC-MS/MS Kit for all major mass spectrometric platforms**
- **Certified reference materials (traceable to the new NIST SRM 972a standard, DGKL ring trial)**
- **Fast and effective 3 – step sample preparation**
- **Removal of proteins and phospholipids from sample**

The Biocrates Vitamin D Kit is characterized by an easy to perform, quick and highly effective matrix-assisted liquid-liquid extraction on a 96-well plate. The 3-step sample preparation removes proteins and phospholipids guaranteeing a longer performance of the analytical column and the robustness of the analysis. After the time-saving sample preparation the extracts are immediately measured. The special design of the analytical column enables to run the analysis under both HPLC and UHPLC conditions.

Employing APCI as ion source and the isotopic labelled internal standard allows to optimise the precision, accuracy and robustness of the kit and to minimise interfering matrix effects at the same time.

**Specifications**
- HPLC run: 5 min
- UHPLC run: 2 min
- Precision: CV intra assay: < 5 % LLOQ: 3 ng/ml
- Accuracy NIST: 98 – 101%

**3-step sample preparation (only 50 µL sample volume needed)**
- Pipette samples and ISTD directly on the extraction plate
- Elute after 5 min with EtOAc
- Dry the extract and resuspend in 40 µl MeOH/H2O

**LC-MS/MS – Parameter**
- Injection volume: 20 µl
- Ionisation: APCI +
- MS/MS-Modus: MRM

Calibrators, internal standards, testmix, quality controls are also available as single products!

**Literature:**

**Differentiation between 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2:**

**LC separation of analytes measured under HPLC conditions**

*Source: Biocrates Life Sciences AG*
## Kit Contents

<table>
<thead>
<tr>
<th>Kit Item</th>
<th>Description</th>
<th>Additional Product Information</th>
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<tbody>
<tr>
<td>4 level serum calibrators</td>
<td>Matrix: human serum 25(OH)-VitD2 and 25(OH)-VitD3: cal 1: c = 5 ng/ml cal 2: c = 10 ng/ml cal 3: c = 25 ng/ml cal 4: c = 50 ng/ml 4 vials, lyophilized</td>
<td>Also available as reagents-only set Biocrates® Vitamin D Calibrators 2 x (4 x 0,5 ml)</td>
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<tr>
<td>Isotopic labelled internal standard (ISTD)</td>
<td>1 vial</td>
<td>Also available as reagents-only set Biocrates® Vitamin D Internal Standards 2 x 1,8 µg</td>
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<tr>
<td>Testmix</td>
<td>For the instrument performance check 1 vial</td>
<td>Also available as reagents-only set Biocrates® Vitamin D Testmix 4 x 1 ml</td>
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<tr>
<td>Multilevel quality controls</td>
<td>Matrix: human serum 2 vials, lyophilized</td>
<td>Also available as reagents-only set Biocrates® Vitamin D Quality Controls 10 x (2 x 0,5 ml)</td>
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<td>USB-drive with acquisition- and quantification methods</td>
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<tr>
<td>SLE plate</td>
<td>For sample preparation</td>
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<tr>
<td>96 deepwell plate</td>
<td>For collecting sample extracts after preparation</td>
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<tr>
<td>Silicon mat cover for plate</td>
<td>Covers the plate after sample preparation</td>
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<tr>
<td>Biocrates® Vitamin D Kit User Manual and Specifications</td>
<td>Digital instruction booklet on how to use the Kit</td>
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<tr>
<td>Biocrates® Vitamin D Column Set</td>
<td>Analytical column for UHPLC/HPLC, UHPLC/HPLC-precolumn</td>
<td>To be ordered separately</td>
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## Ordering Information

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<td>Biocrates® Vitamin D Column Set</td>
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<td>Biocrates® Vitamin D Calibrators</td>
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<td>Biocrates® Vitamin D Internal Standards</td>
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<td>Biocrates® Vitamin D Quality Controls</td>
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<td>Biocrates® Vitamin D Testmix</td>
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This product is not commercially available and will not be offered in the USA, Germany, France, Switzerland, Liechtenstein and UK.