Measurement of 25-Hydroxyvitamin D
with a **novel and unique ELISA** assay

100 % **ALL-IN-ONE**
Simple automation
Since many years, the role of vitamin D in bone and mineral metabolism was recognized in bone-related diseases. Clinical applications of 25-Hydroxyvitamin D measurements were merely related to the diagnosis and monitoring of therapy for rickets (children), osteomalacia, postmenopausal osteoporosis, and renal osteodystrophy. As a result of more recent studies, a link between Vitamin D deficiency and many other diseases is suggested. These include cancer, cardiovascular disease, autoimmune diseases, diabetes, depression.

Clinical aspects:

There are two forms of Vitamin D in the human body namely Vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol), which are structurally very similar. Vitamin D3, the main form in humans, is produced in the skin from 7-dehydrocholesterol in response to direct sunlight and can also be obtained in small amounts from animal-based foods (oily fish, primarily salmon and mackerel). Vitamin D2 can be obtained in small amounts from plant-based foods (some vegetables, yeast and fungi). Vitamin D3 and D2 are metabolized in the liver to their respective 25-Hydroxyvitamin D3 and D2 forms which are converted in the kidneys to the active forms (1,25-dihydroxyvitamin D3 and D2).

Determining Vitamin D Status

The measurement of 25-Hydroxyvitamin D concentration in the serum or plasma is the best indicator of vitamin D nutritional status. It is generally accepted that serum 25-Hydroxyvitamin D levels reflect the body’s storage levels of vitamin D and correlate with the clinical symptoms of vitamin D deficiency. There is no consensus about the optimal 25-Hydroxyvitamin D level, but many publications suggest a range ≥30 ng/mL (>80nmol/L) as optimal. The most widely used intervals are indicated in Table 1.

Paediatric reference intervals have not been established, but the American Association for Paediatrics (AAP) recommends a value of 20 ng/mL for healthy children.

Several population studies have identified widespread 25-Hydroxyvitamin D insufficiency (> 40% of the population) in apparent healthy populations.

About 70 percent of the United States population has inadequate levels of vitamin D. Groups that are at higher risk for inadequate levels of 25 Hydroxyvitamin D levels include, individuals with limited sun exposure, the elderly, dark-skinned individuals and breast-feeding infants.

Measurements of 25-Hydroxyvitamin D levels can be used in:

1. Diagnosing Vitamin D insufficiency or deficiency, to help identifying individuals who may benefit from Vitamin D supplementation to reach optimal levels.
2. Monitoring response to Vitamin D supplements for bone-related diseases e.g. rickets (children), osteomalacia, postmenopausal osteoporosis, and renal osteodystrophy or non-bone related diseases.
3. Diagnosing Vitamin D toxicity, e.g. patients with suspected toxicity (hypercalcemia).
Method principle and Characteristics

The DIAsource® 25-Hydroxyvitamin D Total ELISA is a competitive ELISA with a novel pre-treatment step performed inside the ELISA MT-plate. During a first 2 hours incubation step, at room temperature, 25-Hydroxyvitamin D (D2 and D3) present in calibrators, controls and samples is dissociated from binding serum proteins to fix on binding sites of a specific monoclonal antibody (Patent Pending). After a washing step a fixed amount of 25-Hydroxyvitamin D labelled with biotin in presence of horseradish peroxidase (HRP), competes with unlabelled 25-Hydroxyvitamin D2 and 25-Hydroxyvitamin D3 present on the binding sites of the specific monoclonal antibody. After a 30 minutes incubation at room temperature, the MT-plate is washed to stop the competition reaction.

The Chromogenic solution (TMB) is added and incubated for 15 min. The reaction is stopped with the addition of Stop Solution and the MT-plate is then read at the appropriate wavelength. The amount of substrate turnover is determined colorimetrically by measuring the absorbance, which is inversely proportional to the total 25-Hydroxyvitamin D (D2 and D3) concentration.

Calibration

The DIAsource® 25-Hydroxyvitamin D Total ELISA is calibrated against the reference method ID-LC/MS-MS*

A correlation was performed with 94 serum samples comparing the DIAsource® 25-Hydroxyvitamin D Total ELISA to LC/MS-MS. The regression analysis demonstrated a slope of 0.914, an intercept of 2.33 ng/mL (Figure 1) and a correlation of R= 0.92

This LC/MS-MS methodology used for the correlation studies of the DIAsource® 25-Hydroxyvitamin D Total ELISA assay shows a high level of traceability to the ID-LC/MS-MS reference methodology (Correlation Coefficient R>0.97).

A further correlation was performed with 74 serum samples comparing the DiAsource® 25-Hydroxyvitamin D Total ELISA assay with the commercially available and FDA approved DiaSorin 25-Hydroxyvitamin D Liaison assay. The regression analysis demonstrated a slope of 1.075, an intercept of 0.158 ng/ml (Figure 2) and a correlation of R= 0.93.

In addition to the comparison with DiaSorin Liaison, a correlation was performed in 118 serum samples with the commercially available IDS 25-Hydroxyvitamin D ELISA assay. The regression analysis shows a slope of 1.063, an intercept of 0.831 ng/ml (Figure 3) and a correlation of R=0.93.

Assay performances

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-Reactivity (%)</th>
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<tbody>
<tr>
<td>25OH-Vitamin D3</td>
<td>100</td>
</tr>
<tr>
<td>25OH-Vitamin D2</td>
<td>83</td>
</tr>
<tr>
<td>1,25(OH)2-Vitamin.D3</td>
<td>50</td>
</tr>
<tr>
<td>1,25(OH)2-Vitamin.D2</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Vitamin D2</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>24,25(OH)2-Vitamin.D3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>25,26(OH)2-Vitamin D3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3-epi-25 hydroxy vitamin D3</td>
<td>&lt;0.2</td>
</tr>
</tbody>
</table>

The DiAsource® 25-Hydroxyvitamin D Total ELISA assay demonstrated an excellent recovery: the assay showed an equimolar detection of both abundant 25-Hydroxyvitamin D forms (D2 and D3) in the human body.
The DIAsource® 25-Hydroxyvitamin D Total ELISA assay is a competitive enzyme-immunoassay for the quantitative determination of 25-Hydroxyvitamin D Total (D2 + D3) in human serum. It makes use of a novel pre-treatment step which eliminates the use of solvent precipitation and centrifugation. This sample pre-treatment step is performed inside the sample well of the ELISA microtiter plate which highly facilitates the automation on different open ELISA platforms.

**Assay Protocol**

1. **add 50 µl sample, Standard Control** and add 150 µL Incubation Buffer
2. **Incubate 120 min at RT with shaking**
3. **Wash Plate**
4. **Incubate 30 min at RT with shaking**
5. **Wash Plate**
6. **add 100 µL TMB**
7. **Incubate 15 min at RT with shaking**
8. **add 100 µL STOP**
9. **Read Absorbance at 450 nm**

The novel approach of the DIAsource® 25-Hydroxyvitamin D Total ELISA assay, makes the assay easy to program on any open ELISA platform without the need for manual sample pre-treatment outside the ELISA platform.

The DIAsource® 25-Hydroxyvitamin D Total ELISA has extensively been validated on the Stratec® Gemini. A validated protocol is available and permits larger laboratories to easily automate their 25-Hydroxyvitamin D determinations.

A in-house correlation was performed with 54 serum samples comparing the DIAsource® 25-Hydroxyvitamin D Total ELISA assay manually performed and automated on the Stratec® Gemini, an OPEN ELISA automate. The regression analysis demonstrated a slope of 0.98, an intercept of –2.29 ng/ml and a correlation of $R = 0.98$. 

**Automation : Application on open ELISA platform Stratec® Gemini**

$y = 0.9811x - 2.2918$

$R = 0.98$
**DIAsource® 25-Hydroxyvitamin D Total ELISA (art. code KAP1971)** assay shows very competitive sensitivity, precision and performance characteristics to all other immunoassays in the market but uses a novel and extremely easy pre-treatment step directly in the microtiter plate.

The assay is calibrated to the ID-LC/MS-MS reference method. It shows a high correlation with other commercially available Chemiluminescence assays, with LC/MS-MS and with other 25-hydroxyvitamin D ELISA assays.

The novel approach of the DIAsource® assay, makes it extremely easy to program on any open ELISA platform without the need for a manual sample pre-treatment outside the ELISA automate as is the case in other commercially available ELISA assays.

A validated assay protocol is available on the Stratec® Gemini open ELISA automate and permits larger laboratories to easily automate their 25-hydroxyvitamin D determinations.

**Ordering Information:**

<table>
<thead>
<tr>
<th>Article</th>
<th>Description</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAP1971</td>
<td>25-Hydroxyvitamin D Total ELISA</td>
<td>96 test</td>
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</tbody>
</table>

*AIO = ALL IN ONE*, means that pre-treatment and ELISA are performed in the same MT-plate, which does not require a pre-treatment step in a second MT-plate or tubes before to transfer pre-treated samples to the MT-plate.