PROTOCOL: ALL-IN-ONE PRINCIPLE

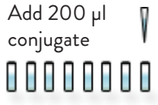
The DIAsource 25OH Vitamin D Total ELISA assay makes use of a novel pre-treatment step. This sample pre-treatment step is performed inside the sample well of the ELISA microtiter plate which highly facilitates the automation on different open ELISA platforms.

ASSAY PROTOCOL : MOST CONVENIENT ASSAY ON THE MARKET

Add 50 µL sample, Standard, control and add 150 µL Incubation Buffer in the MT well

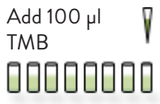
Incubate 120 min at RT with shaking → Wash plate

Add 200 µl conjugate



Incubate 30 min at RT with shaking → Wash plate

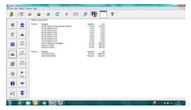
Add 100 µl TMB



Incubate 15 min at RT with shaking →

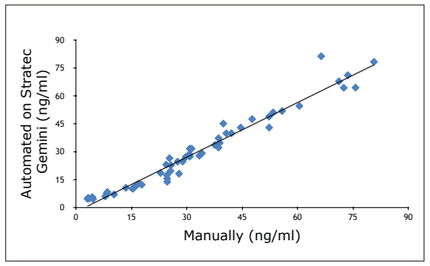
Add 100 µl STOP

Read Absorbance at 450 nm →



AUTOMATION: VALIDATED APPLICATION ON STRATEC GEMINI OPEN ELISA PLATFORM

The DIAsource 25OH Vitamin D Total ELISA has extensively been validated on the Stratec Gemini. A validated protocol is available and permits larger laboratories to easily automate their 25OH Vitamin D determinations. An in-house correlation was performed with 54 serum samples comparing the DIAsource 25OH Vitamin 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.



Ordering information

Description	Article code	Format	Size
25OH Vitamin D Total ELISA	KAP1971	ELISA	96 tests
OTHER DIASOURCE BONE METABOLISM ASSAYS			
Free 25OH Vitamin D ELISA RUO	KARF1991	ELISA	96 tests
1,25$(OH)_2$ Vitamin D ELISA	KAP1921	ELISA	96 tests
1,25$(OH)_2$ Vitamin D Extraction Cartridges	1102496	For KAP1971	1 bag of 42 cartridges
1,25 $(OH)_2$ Vitamin Extraction kit (Solvents), ready to use	3019700	For KAP1971	2 x 42 extractions
Intact PTH ELISA	KAP1481	ELISA	96 tests
Osteocalcin ELISA	KAP1381	ELISA	96 tests
Aggrecan (PG – Proteoglycan) ELISA	KAP1461	ELISA	96 tests

For more information: visit www.diasource-diagnostics.com

Manufactured by: DIAsource ImmunoAssays SA
Rue du Bosquet 2
BE 1348 Louvain-La-Neuve
Tel. 32 10 84 99 00
Fax 32 10 84 99 96

Distributed by:

LIT3021-1505

25OH VITAMIN D TOTAL ELISA ASSAY

Novel and UNIQUE ELISA assay.

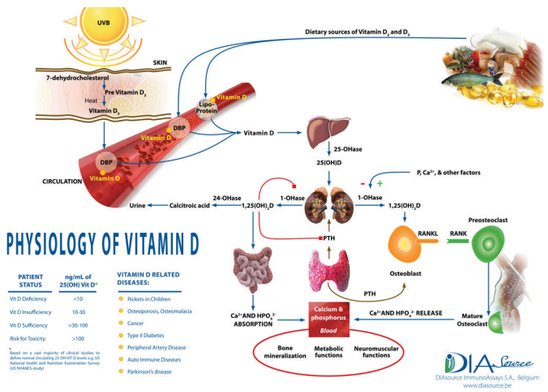
100% ALL-IN-ONE Simple automation.

The most user friendly 25OH Vitamin D Total ELISA assay.

Since many years the role of vitamin D in bone and mineral metabolism was recognized in bone-related diseases. Clinical applications of 25OH Vitamin D measurements were merely related to the diagnosis and monitoring of therapy for rickets (children), osteomalacia, postmenopausal osteoporosis, and renal osteodystrophy. As a results 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 and many others.

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 UVB 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). Both forms are available as supplements, the D3 form remaining the most current in many regions. Vitamin D3 and D2 are metabolized in the liver to their respective 25OH Vitamin D3 and D2 forms which are converted in the kidneys and other tissues to the active forms ($1.25(OH)_2$ Vitamin D3 and D2).



DETERMINING VITAMIN D STATUS

The measurement of the 25OH Vitamin D concentration in serum or plasma is so far the best indicator of Vitamin D nutritional status. It is generally accepted that serum 25OH Vitamin 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 25OH Vitamin 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.

Several population studies have identified widespread 25OH Vitamin D insufficiency (> 40% of the population) in apparent healthy populations. Paediatric reference intervals have not been established, but the American Association for Paediatrics (AAP) recommends a value of 20 ng/mL for healthy children.

MEASUREMENT OF 25OH VITAMIN 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).

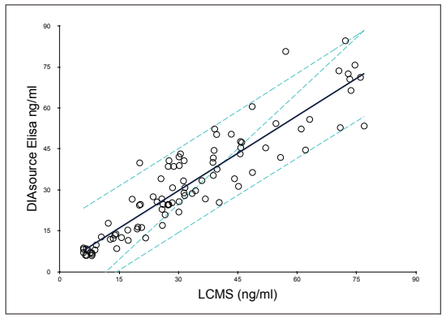
METHOD PRINCIPLE AND SPECIFICITY

The DIAsource 25OH Vitamin D Total ELISA uses patented Monoclonal antibody, proprietary biotinylated antigen and a unique displacement reagent. The DIAsource 25OH Vitamin D Total ELISA assay demonstrated very low cross-reactivity to other metabolites of Vitamin D3 and D2. The assay performance is not affected by hemolysis, bilirubinemia, Vitamin C, biotin or triglycerides.

Table 1: Suggested Reference Values for Adults	
Vitamin D Status	25OH Vitamin D Total (ng/mL)
Deficiency	< 10
Insufficiency	10 - 29
Sufficiency	30 - 100
Toxicity	> 100

25OH Vitamin D Total ELISA (KAP 1971)	
Art. Code	KAP1971
Format	ELISA breakable wells
Size	96 tests
Sample Type	Serum
Sample Volume	50 µL
Controls	2 levels
Range	0-180 ng/mL
LoD	2.8 ng/mL
LoQ	4.3 ng/mL
Total Analysis Time	< 4 hours
Incubation	120' + 30' + 15'
Wavelength	450 nm

Compound	Cross-Reactivity (in %)
25OH Vitamin D3	100
25OH Vitamin D2	83
$1,25(OH)_2$ Vitamin D3	20
$1,25(OH)_2$ Vitamin D2	1,9
Vitamin D3	2,9
Vitamin D2	1,3
$24,25(OH)_2$ Vitamin D3	> 100
$25,26(OH)_2$ Vitamin D3	> 100
3-epi-25OH	0,1



CALIBRATION

The DIAsource 25OH Vitamin D Total ELISA is calibrated against the reference method ID-LC-MS/MS. This LC-MS/MS methodology used for the correlation studies of the DIAsource 25OH Vitamin D Total ELISA assay shows a high level of traceability to the ID-LC/MS-MS reference methodology used in the Vitamin D Standardisation Program VDSP (Correlation Coefficient R>0.97).

A correlation was performed with 94 serum samples comparing the DIAsource 25OH Vitamin D Total ELISA to LC-MS/MS. The regression analysis demonstrated a slope of 0.91, an intercept of 2,33 ng/mL and a correlation of R= 0.92.

METHOD COMPARISON

Multiple correlation studies were performed. A summary is listed in table 1.

Assay	Place	Regression	R
LC-MS/MS	DIAsource – Manchester (UK)	0.91 X + 2.33 ng/mL	0.92
IDS ELISA	DIAsource	1.06 X + 0.83 ng/mL	0.93
IDS ELISA	US (3 sites)	0.95 X + 3.05 ng/mL	0.96
DiaSorin Liaison	DIAsource – Nice (Fr)	1.13 X - 1.34 ng/mL	Passing & Bablok*
DiaSorin Liaison	Nice (Fr)	0.98 X + 1.94 ng/mL	0.94
Euroimmun	DIAsource – Nice (Fr)	1.02 X - 2.14 ng/mL	Passing & Bablok*
DRG	DIAsource – Nice (Fr)	1.19 X + 2.28 ng/mL	Passing & Bablok*
DRG	DRG – Nice (Fr)	1.24 X + 1.12 ng/mL	Passing & Bablok*

Table 1 : summary data of in-house and external performed correlation studies

* Passing & Bablok regression procedure makes no special assumptions regarding the distribution of the samples and the measurement errors and does not provide a correlation coefficient R.

PROTOCOL COMPARISON

	DIAsource (art. Code KAP1971)	IDS Ltd (ELISA)	Euroimmun (ELISA)	DRG (ELISA)
Pretreatment All-In-One	In the assay well	Outside the assay well (Sample dilution in tubes)	Outside the assay well (Sample dilution in tubes)	Outside the assay well (Sample dilution in tubes)
Transfer Pre-treated sample into MT	NOT NECESSARY	YES	YES	YES
100 % Automatable	YES	NO	NO	NO
Specificity	100% D3 & 86% D2	100% D3 & 75% D2	100% D3 & 100% D2	DBP -assay
Vortex	NO	Each sample 10 sec. (pre-treatment)	NO	Each sample 10 sec. (pre-treatment)
Analytical sensitivity	2,8 ng/ml	2,0 ng/ml	2,2 ng/ml	
N° of steps	6	10	20	15
Total Incubation time	120' + 30' + 15'	120' + 30' + 30'	10' + 120' + 30' + 15'	30' + 60' (37°C) + 15'
Total Analysis time	< 210'	> 210'	> 210'	< 210'

PRACTICAL ADVANTAGES OF THE DIASOURCE 25OH VITAMIN D TOTAL ELISA ASSAY

The DIAsource 25OH Vitamin D Total ELISA 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 25OH vitamin 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 .