

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

- **100 % ALL-IN-ONE**
- **Simple automation**



Unique assay on the market!



The DIAsource 25-Hydroxyvitamin D Total ELISA is powered by patented and unique DIAsource monoclonal antibodies that express 100 % reactivity against 25 OH Vitamin D3 and 83% reactivity against 25OH Vitamin D2.

They make the assay unique in the market and will guarantee the highest lot-to-lot reliability and consistency.

- ü All In One technology
- ü No extraction step, displacement solution directly into the well
- ü Results in less than 4 hours
- ü Calibrated against ID-LC/MS-MS (reference method)
- ü Validated on the Stratec® GEMINI open ELISA automate (Stratec Biomedical GMBH – Germany) and offers a validated protocol to all Stratec® GEMINI users.
- ü Bar codes on all components



Method principle

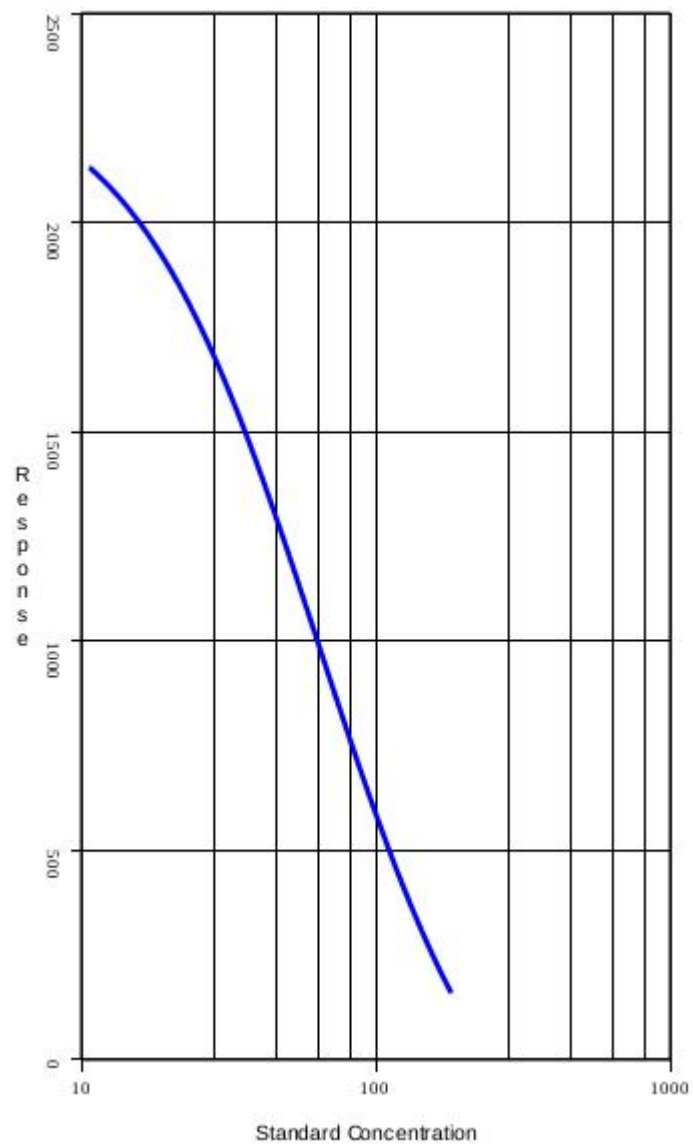
The DIAsource® 25-Hydroxyvitamin D Total ELISA is a competitive ELISA with a novel pre-treatment step performed inside the ELISA MT-plate. During a first 2 hours incubation step, at room temperature, 25-Hydroxyvitamin D (D2 and D3) present in calibrators, controls and samples is dissociated from binding serum proteins to fix on binding sites of a specific monoclonal antibody (DIAsource patented). 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 .



Assay characteristics



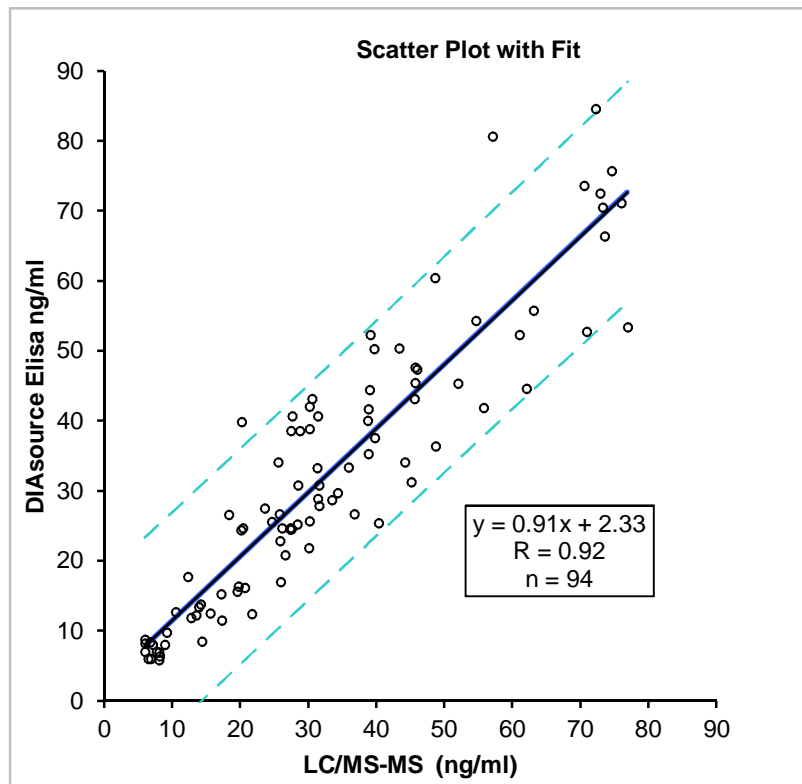
25OH Vitamin D Total ELISA	
Cat #:	KAP1971
Format:	ELISA MT
Size:	96 Tests
Sample Type:	Serum
Sample Volume:	50 μL
Controls:	2 levels
Range:	0-180 ng/mL
Sensitivity:	1,4 ng/mL
Total Incubation Time	< 4 hours
Protocol steps	120/30/15 min.
Wavelength	450 nm



Assay 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 0.91, an intercept of 2,33 ng/mL and a correlation of R= 0.92

* *Candidate Reference Measurement Procedures for Serum 25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D2 by Using Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry (ID-LC/MS-MS), Clinical chemistry, 2011, Vol.57,3, pag 441 - 44*

Easy to Use protocol!



Calibrators/Controls/Samples	50 μL
Incubation Buffer	150 μL
Incubate 2 hours at room temperature with shaking	
Aspirate and wash 3 times	
Diluted conjugate	200 μL
Incubate 30 min. at room temperature with shaking	
Aspirate and wash 3 times	
Ready to Use TMB	100 μL
Incubate 15 min. at room temperature with shaking	
Stop Solution	100 μL

Specificity



The percentage of cross reaction estimated by comparison of the concentration yielding a 50 % inhibition are respectively :

Coumpound	Cross-Reactivity (%)
25OH-Vitamin D ₃	100 %
25OH-Vitamin D ₂	83%
1,25(OH) ₂ -Vitamin D ₃	50%
1,25(OH) ₂ -Vitamin D ₂	<0,2%
Vitamin D ₃	<0,2%
Vitamin D ₂	<0,2%
24,25(OH) ₂ -Vitamin D ₃	>/= 100%
25,26(OH) ₂ -Vitamin D ₃	>/= 100%
3-epi-25 hydroxyvitamin D ₃	<0,2%

The assay performance is not affected by hemolysis (5g/L hemoglobin tested), bilirubinemia (0,5g/L bilirubin tested) or triglycerides (5g/L tested).

Precision



Sample	INTRA-ASSAY			INTER-ASSAY		
	N	<x> +/- SD (ng/mL)	CV (%)	N	<x> +/- SD (ng/mL)	CV (%)
A	35	27,4 +/- 1,5	5,5	10	26,3 +/- 1,3	4,9
B	35	43,0 +/- 1,2	2,7	10	42,0 +/- 1,9	4,5

Accuracy (recovery)



RECOVERY TEST	
Added 25OH-Vit D3 (ng/mL)	Recovery (%)
0	100
25	95
50	92
Added 25OH-Vit D2 (ng/mL)	Recovery (%)
0	100
25	105
50	95

Accuracy (dilution test)



DILUTION TEST			
Sample Dilution	Theoretical concent. (ng/mL)	Measured concent. (ng/mL)	Recovery (%)
1/1	66,2		
1/2	33,1	34,5	104
1/4	16,5	15,5	93
1/8	8,2	8,2	99
1/16	4,1	4,4	106
1/32	2,1	2,2	106
1/1	62,0		
1/2	31,0	38,3	123
1/4	15,5	15,8	102
1/8	7,7	7,5	97
1/16	3,8	4,0	103

Expected Values

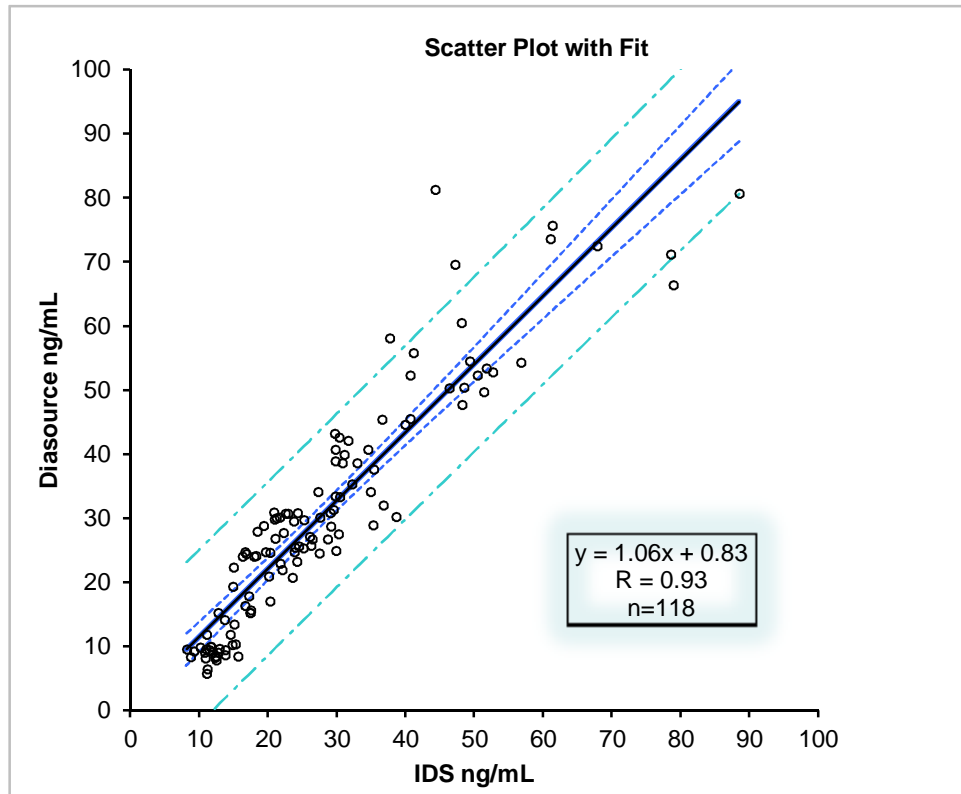


Dietary intake, race, season and age are known to affect the normal levels of 25OH.Vit.D3. Each laboratory should establish its own range based on their local population.

Recent literature has suggested the following ranges for the classification of 25 OH Vitamin D status: Deficiency: 0-10 ng/mL; Insufficiency: 10-30 ng/mL; Sufficiency: 30 to 150 ng/mL; Toxicity: >150 ng/mL.



Correlation with IDS ELISA



A correlation was performed with 118 serum samples comparing the DIAsource® 25-Hydroxyvitamin D Total ELISA to the IDS ELISA assay. The regression analysis demonstrated a slope of 1.06, an intercept of 0.83 ng/mL and a correlation of $R = 0.93$

Protocol comparison with IDS ELISA



	DIAsource	IDS
Pretreatment	None	25 µL of sample, calibrator and control + 1mL of Biotin Solution into a glass or polypropylene tube
		Vortex
Calibrators/Controls/Samples	50 µL	Add 200 µL of diluted sample in the appropriate wells
Incubation Buffer	150 µL	
Incubation	Incubate 2 hours at room temperature with shaking	Incubate 2 hours at room temperature
Washing	Aspirate and wash 3 times	Aspirate and wash 3 times
Diluted conjugate	200 µL	200 µL
Incubation	Incubate 30 min. at room temperature with shaking	Incubate 30 min. at room temperature
Washing	Aspirate and wash 3 times	Aspirate and wash 3 times
Ready to Use TMB	100 µL	200 µL
Incubation	Incubate 15 min. at room temperature with shaking	Incubate 30 min. at room temperature
Stop Solution	100 µL	100 µL
Total turnaround time	2h45 min.	3h30 min.