

REFERENCE VALUES

These values are given only for guidance (reference values dependent of the season). The observed ranges are based on 2.5% to 97.5% percentiles.

Population	Range (pg/mL)	Mean	SD	N
Healthy Adults	19.6-54.3	35.3	10.6	51

PRACTICAL ADVANTAGES OF THE DIASOURCE 1,25(OH)₂ VITAMIN D ELISA ASSAY - TIMELINE

The timetable represent a graphic overview of the protocol handling time. One can notice that the DIAsource 1,25(OH)₂ Vitamin D RIA assay has the fastest TAT (Turn Around Time) compared with 2 other commercially available RIA assays.

Hour of the day	9	10	11	12	13	14	15	16	17	18	19	20	21	22
RIA Kit DIAsource														
RIA Kit Competitor A														
RIA Kit Competitor B														

16:00h - 10:00h overnight incubation ☐

- Extraction of sample
- Isolation of 1,25(OH)₂ Vitamin D by specific separation technique
- RIA assay incubation
- RIA assay separation and absorbance reading

DIASOURCE 1,25(OH)₂ VITAMIN D RIA: KEY FEATURES

- Gold Standard extraction protocol ensuring no interference
- Limited number of incubation steps
- The ONLY Coated Tube format available on the market
- Close to 100% cross-reactivity against 1,25(OH)₂ Vitamin D2
- Very low Limit of Detection (1,4 pg/mL)
- Good correlation with LC-MS/MS
- DEQAS samples accurately measured
- No use of Nitrogen evaporation

Ordering information	Description	Article code	Format	Size
	1,25(OH) ₂ Vitamin D RIA	KIP1929	RIA	96 wells
	1,25(OH) ₂ Vitamin D Extraction Cartridges	1102491	For KIP1929	1 bag of 20 cartridges
	1,25(OH) ₂ Vitamin D Extraction Kit (Solvents, ready-to-use)	3019700	For KIP1929	5 x 48 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

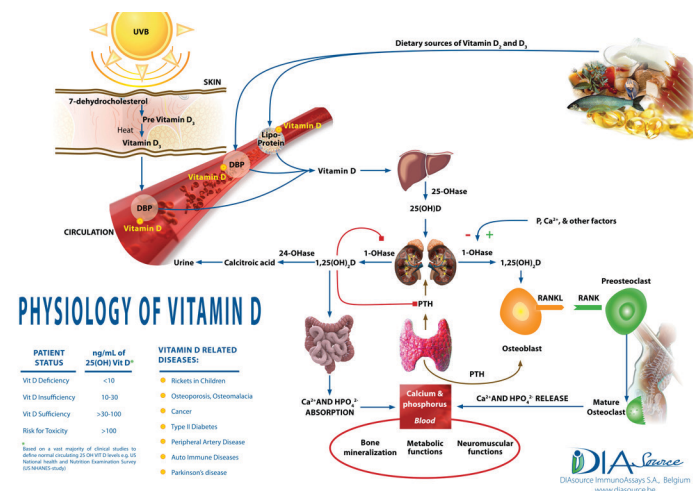
Distributed by:



1,25(OH)₂ VITAMIN D RIA ASSAY



1,25(OH)₂ Vitamin D assessment with coated tube technology and Gold Standard Extraction method.



CLINICAL ASPECTS

Vitamin D is mainly synthesized in the skin from 7-dehydrocholesterol and is partially from dietary origin. In the liver, Vitamin D is hydroxylated on carbon 25 to produce the obligatory intermediate 25OH Vitamin D. 25OH Vitamin D must be metabolized further before it can carry out the functions of Vitamin D on intestine, kidney and bone. This subsequent reaction takes place in the kidney (endocrine actions) and in other organs and tissues (paracrine/autocrine actions). Thus 25OH Vitamin D is further hydroxylated in the 1 α -position to produce 1 α ,25 dihydroxyvitamin D (1,25(OH)₂ Vitamin D).

1,25(OH)₂ Vitamin D is the active form of Vitamin D with regard to the known functions whereas 25OH Vitamin D and Vitamin D itself can be excluded as being physiologically

functional. Furthermore since 1,25(OH)₂ Vitamin D is produced in the kidney and has some of its functions in the intestine, kidney, bone and other organs and tissues, it must be considered as a hormone. This hormone stimulates the intestinal absorption of both calcium and phosphorus. It also stimulates bone resorption and mineralization thereby preventing the development of rickets and osteomalacia.

The levels of 1,25(OH)₂ Vitamin D in plasma or serum is 100 to 1000 less than that of 25OH Vitamin D. Due to its low concentrations and the presence of many similar metabolites, the measurement of 1,25(OH)₂ Vitamin D requires extraction and separation either by HPLC or by column chromatography preferable with the use of organic solvent. This extraction method is still recognized as the Gold Standard for 1,25(OH)₂ Vitamin D.

Graham D Carter (DEQAS Organizer):

The successive abandonment of sample extraction, chromatography, [...] in immunoassays has undoubtedly contributed to the inconsistencies reported by Farrell et al., [...].

25-Hydroxyvitamin D: A Difficult Analyte. Clinical Chemistry 2012, 58:3486–488.

CLINICAL APPLICATION

The measurement of circulating 1,25(OH)₂ Vitamin D is indicated in several disorders affecting calcium metabolism such as:

- Assessment of Kidney functions: chronic kidney failure and Haemodialysis (after kidney transplants)
- Hyper and hypo-parathyroidism,
- Rickets disease
- Tumor-associated hypercalcemia
- Osteomalacia as a results of various types of Vit D metabolism disturbances

METHOD PRINCIPLE AND CHARACTERISTICS

Only samples and controls, not the calibrators, are extracted with a mix of solvents and applied on cartridges to separate 1,25(OH)₂ Vitamin-D from other Vitamin-D metabolites. After elution of samples and controls, the calibrators, samples and controls are incubated in coated tubes. A fixed amount of 125I labelled 1,25(OH)₂ Vitamin D competes with the 1,25(OH)₂ Vitamin D to be measured present in the sample or in the calibrator for a fixed amount of antibody sites immobilized on the wall of the coated tube. After an overnight incubation at room temperature, an aspiration step terminates the competition reaction. The tubes are then washed with washing solution and aspirated. A calibration curve is plotted and the 1,25(OH)₂ Vitamin D concentrations of the samples are determined by dose interpolation from the calibration curve.

1,25(OH) ₂ Vitamin D RIA	
Art. Code	KIP1929
Format	RIA CT
Label	I125
Size	48 tests
Sample Type	Serum, Plasma
Sample Volume	500 μ L
Controls	2 levels
Range	4.7-350 pg/mL
Sensitivity	1.4 pg/mL
Incubation	Overnight
Shelf Life (weeks)	10

SPECIFICITY

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

Compound	Cross-Reactivity (in %)
1,25(OH) ₂ Vitamin D3	100
1,25(OH) ₂ Vitamin D2	92.3
25OH Vitamin D3	0.001
24,25(OH) ₂ Vitamin D3	0.005
25,26(OH) ₂ Vitamin D3	0.20

ACCURACY: DILUTION TEST (sample was diluted with Elution solution)

Sample Dilution	Theoretical Conc. (pg/mL)	Measured Conc. (pg/mL)	Recovery (%)
1/1	70,0	70	100
1/2	35,0	35.7	102
1/4	17.5	14.5	83
1/8	8.8	7.8	89
1/16	4.4	4.6	105
		Mean Recovery	95.8

ACCURACY: RECOVERY TEST

Added 1,25(OH) ₂ Vitamin D (pg/ml)	Measured 1,25(OH) ₂ Vitamin D (pg/ml)		Recovery (%)
	Total	Blanked	
0.0	22.5		
25.0	46.3	23.8	95.2
50.0	70.0	47.5	95.0
100	122.7	100.2	100.2
Added 1,25(OH) ₂ Vitamin D (pg/ml)	Measured 1,25(OH) ₂ Vitamin D (pg/ml)		Recovery (%)
	Total	Blanked	
0.0	22.5		
25.0	52.1	29.6	118.4
50.0	70.4	47.9	95.8
100	112.9	90.4	90.4

ASSAY COMPARISON

	DIAsource RIA	IDS Gamma RIA	DiaSorin RIA
Hands-on-time	3,5 hrs	3,5 hrs	3,5 hrs
RIA coated tube	YES	NO	NO
Vacuum needed	NO	YES	YES
N ₂ needed	NO	YES	YES
Sample volume needed	500 μ L	500 μ L	500 μ L
Cartridges included in kit	YES	YES	NO
Nb of samples per kit	19 (38 with second bag of cartridges)	2 versions: 20 and 28	24