



25OH-Vitamin D total-RIA-CT

KIR1971

For Informational/Research Purposes Only

History

Summary of change:

Previous Version: 200430-1				Current Version: 200615			
V. REAGENTS PROVIDED				V. REAGENTS PROVIDED			
Reagents	96 Tests Kit	Colour Code	Reconstitution	Reagents	96 Tests Kit	Colour Code	Reconstitution
[Ag 125I] 125Iodine labelled 25OH Vit D (HPLC grade).	1 vial 160 kBq lyophilised	red	Add 6 ml of Tracer Buffer	[Ag 125I] 125Iodine labelled 25OH Vit D (HPLC grade).	1 vial 168 kBq lyophilised	red	Add 10.5 ml of Tracer Buffer
[TRACER BUF] Tracer Buffer with casein, gentamycin and red dye	1 vial 7 ml	red	Ready for use	[TRACER BUF] Tracer Buffer with casein, gentamycin and red dye	1 vial 11.5 ml	red	Ready for use
VI. SUPPLIES NOT PROVIDED 2. Pipettes for delivery of: 25 µl, 50 µl, 500 µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)				VI. SUPPLIES NOT PROVIDED 2. Pipettes for delivery of: 25 µl, 100µl, 500 µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)			
VII. REAGENT PREPARATION C. Tracer: Reconstitute the lyophilised tracer with 6 ml of the Tracer Buffer.				VII. REAGENT PREPARATION C. Tracer: Reconstitute the lyophilised tracer with 10.5 ml of the Tracer Buffer.			
X. PROCEDURE 5. Dispense 50 µl of ¹²⁵ Iodine labelled 25OH Vitamin D into each tube, including the uncoated tubes for total counts.				X. PROCEDURE 5. Dispense 100 µl of ¹²⁵ Iodine labelled 25OH Vitamin D into each tube, including the uncoated tubes for total counts.			
XII. TYPICAL DATA				XII. TYPICAL DATA			
25OH Vitamin D total		cpm	B/Bo (%)	25OH Vitamin D total		cpm	B/Bo (%)
Total count		52033		Total count		67320	
Calibrator	0.0 ng/ml	17721	100.0	Calibrator	0.0 ng/ml	20520	100.0
	10 ng/ml	11022	62.2		5.8 ng/ml	16288	79.4
	20 ng/ml	6826	38.5		13 ng/ml	10274	50.0
	40 ng/ml	3446	19.4		35 ng/ml	6398	31.2
	60 ng/ml	1469	8.3		50 ng/ml	3926	19.1
	100 ng/ml	592	3.3		100 ng/ml	1190	5.8

XIII. PERFORMANCE AND LIMITATIONS

A. Detection Limit

The LOB (Limit of Blank) was calculated by measuring the blank several times and was calculated as the mean - 1.65 Standard Deviation of the distribution of these values. The LOB was calculated to be 1.2 ng/ml. The LOD (limit of detection) was calculated as the LOB - 1.65 Standard Deviation of a low concentration sample tested in 10 different runs. The LOD was calculated to be 5.67 ng/ml. The LOQ (Limit of Quantitation) was calculated by testing 5 samples of low values 10 times. The LOQ was calculated to be 7 ng/ml.

B. Specificity

Compound	Cross-Reactivity (%)
25OH-Vitamin D ₃	100
25OH-Vitamin D ₂	86
1,25(OH) ₂ -Vitamin.D ₃	2.6
1,25(OH) ₂ -Vitamin.D ₂	2.1
Vitamin D ₃	0.8
Vitamin D ₂	0.1
3-epi-25 hydroxy Vitamin D ₃	0.4
24,25(OH) ₂ -Vitamin.D ₃	≥100
25,26(OH) ₂ -Vitamin D ₃	≥100

The assay performance is not affected by hemolysis (5 g/L hemoglobin tested) and by bilirubinemia (0.5 g/L bilirubin tested) [...]

C. Precision

INTRA-ASSAY			
Sample	N	<X> ± SD (ng/ml)	C.V.(%)
A	18	13.2 ± 0.8	5.9
B	18	28.5 ± 0.9	3.3

INTER-ASSAY			
Sample	N	<X> ± SD (ng/ml)	C.V.(%)
A	13	15.1 ± 1.1	7.4
B	13	30.3 ± 1.5	4.9

XIII. PERFORMANCE AND LIMITATIONS

A. Detection Limit

The LOB (Limit of Blank) was calculated by measuring the blank several times and was calculated as the mean + 1.65 Standard Deviation of the distribution of these values. The LOB was calculated to be 0.8 ng/ml. The LOD (limit of detection) was calculated as the LOB + 1.65 Standard Deviation of a low concentration sample tested in 10 different runs. The LOD was calculated to be 1.9 ng/ml. The LOQ (Limit of Quantitation) was calculated by testing 5 samples of low values 10 times. The LOQ was calculated to be 2.6 ng/ml.

B. Specificity

Compound	Cross-Reactivity (%)
25OH-Vitamin D ₃	100
25OH-Vitamin D ₂	85
1,25(OH) ₂ -Vitamin.D ₃	4.1
1,25(OH) ₂ -Vitamin.D ₂	0.2
Vitamin D ₃	ND
Vitamin D ₂	0.1
3-epi-25 hydroxy Vitamin D ₃	0.4
24,25(OH) ₂ -Vitamin.D ₃	23
25,26(OH) ₂ -Vitamin D ₃	26.5

ND: Non detectable

The assay performance is not affected by hemolysis (5 g/L hemoglobin tested) and by bilirubinemia (1 g/L bilirubin tested) [...]

C. Precision

INTRA-ASSAY			
Sample	N	<X> ± SD (ng/ml)	C.V.(%)
A	20	23.1 ± 1.1	4.7
B	20	37.1 ± 1.7	4.7

INTER-ASSAY			
Sample	N	<X> ± SD (ng/ml)	C.V.(%)
A	12	21.0 ± 1.4	6.7
B	12	36.6 ± 2.1	5.8

D. Accuracy

RECOVERY TEST

Added 25OH-Vit.D ₃ (ng/ml)	Recovery (%)
14.8	110
45.2	105
Added 25OH-Vit.D ₂ (ng/ml)	Recovery (%)
11.6	102
18.6	113

DILUTION TEST

Sample dilution	Theoretical concent. (ng/ml)	Measured concent. (ng/ml)
1/1	45.1	45.1
1/2	22.5	24.9
1/4	11.3	13.9
1/1	34.5	34.5
1/2	17.2	17.9
1/4	8.6	9.8

E. Time delay between last calibrator and sample dispensing

TIME DELAY

	0 minute (ng/ml)	20 minutes (ng/ml)	30 minutes (ng/ml)
Sample 1	8.9	7.9	8.9
Sample 2	23.4	21.8	20.5
Sample 3	36.5	35.7	37.5

XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNT S µl	CALIBRATOR S µl	SAMPLE (S) CONTROL S µl
Trace r	50	50	50

"Room temperature" or "room temperature (24±4°C)"

D. Accuracy

RECOVERY TEST

Added 25OH-Vit.D ₃ (ng/ml)	Recovery (%)
25.4	82
14.3	81
7.8	104
Added 25OH-Vit.D ₂ (ng/ml)	Recovery (%)
13.8	92
9.0	85
4.2	81

DILUTION TEST

Sample dilution	Theoretical concent. (ng/ml)	Measured concent. (ng/ml)
1/1	95.1	95.1
1/2	47.6	43.1
1/4	23.8	24.3
1/1	61.8	61.8
1/2	30.9	31.7
1/4	15.4	14.0
1/1	76.8	76.8
1/2	38.4	37.9
1/4	19.2	17.9

E. Time delay between last calibrator and sample dispensing

TIME DELAY

	0 minute (ng/ml)	20 minutes (ng/ml)	30 minutes (ng/ml)
Sample 1	12.2	8.9	9.7
Sample 2	27.9	31.6	28.6
Sample 3	44.2	45.6	45.3

XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNT S µl	CALIBRATOR S µl	SAMPLE (S) CONTROL S µl
Trace r	100	100	100

Room temperature (18-25°C)

Read entire protocol before use.

25OH Vitamin D total -RIA-CT

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of 25-hydroxyvitamin D3 and D2 (25-OH-D3 and 25-OH-D2) in serum.

For research use only. Not for use in diagnostic procedures.

II. GENERAL INFORMATION

A. **Proprietary name :** DIAsource 25OH Vitamin D total -RIA-CT Kit

B. **Catalog number :** KIR 1971 : 96 tests

C. **Manufactured by :** DIAsource ImmunoAssays S.A.

Rue du Bosquet 2 , 1348 Louvain-La-Neuve , Belgium

For technical assistance or ordering information contact :

Tel : +32 (0) 10 84 99 00 Fax : +32 (0) 10 84 99 90

For technical assistance or ordering information in the United States contact :

Immuno-Biological Laboratories, Inc. (IBL-America)

Tel : 1-888-523-1246 Fax : 1-763-780-2988 Email : info@ibl-america.com

III. CLINICAL BACKGROUND

Vitamin D is the generic term used to designate Vitamin D3 or cholecalciferol and Vitamin D2 or ergocalciferol. Humans naturally produce Vitamin D3 when the skin is exposed to ultraviolet sun rays.

In the liver mainly, Vitamin D3 is metabolised into 25-Hydroxyvitamin D3 (25 OH D3) which is the main form of Vitamin D circulating in the body.

25 OH D3 is a precursor for other Vitamin D metabolites and has also a limited activity by itself.

The most active derivative is 1,25-Hydroxyvitamin D3, produced in the kidney (or placenta) by 1 α -hydroxylation of 25OHD3.

25OHVitamin D stimulates the intestinal absorption of both calcium and phosphorus and also bone resorption and mineralisation.

25OH Vitamin D might also be active in other tissues responsible for calcium transport (placenta, kidney, mammary gland...) and endocrine gland (parathyroid glands, beta cells...).

Vitamin D3 and Vitamin D2 are also available by ingestion through food or dietary supplementation.

As Vitamin D2 is metabolised in a similar way to vitamin D3, both contribute to the overall Vitamin D status of an individual.

It is the reason why it is very important to measure both forms of 25 OH Vitamin D equally for a correct diagnosis of Vitamin D deficiency, insufficiency or intoxication.

Vitamin D deficiency is an important risk factor for rickets, osteomalacia, senile osteoporosis, cancer and pregnancy outcomes.

The measurement of both 25 OH Vitamin D forms is also required to determine the cause of abnormal serum calcium concentrations in specimen.

Vitamin D intoxication has been shown to cause kidney and tissue damages.





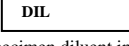


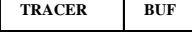
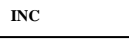
IV. PRINCIPLES OF THE METHOD

At first calibrators, controls and samples (serum) are incubated with the incubation buffer, directly in coated tubes for 2 hours at room temperature (18-25°C), on a shaker, to release 25OH Vitamin D₃ and 25OH Vitamin D₂ from Vitamin D Binding Protein (DBP).

Then, without washing steps, a fixed amount of ¹²⁵I labelled 25OH Vitamin D is added in each tube to compete with the 25OH Vitamin D₃ and 25OH Vitamin D₂ from samples, controls or calibrators, for a fixed amount of a specific monoclonal antibody sites immobilized to the lower and inner surface of plastic tubes.

After 1 hour incubation at room temperature (18-25°C) on a tube shaker, an aspiration step terminates the competition reaction. The tubes are then washed twice and aspirated again. A calibration curve is plotted and the total 25 OH Vitamin D (D₃ and D₂) concentrations of the samples are determined by dose interpolation from the calibration curve.

V. REAGENTS PROVIDED

Reagents	96 Tests Kit	Colour Code	Reconstitution
 Tubes coated with Mab anti 25OH Vit D3 and D2	2 x 48	pink	Ready for use
 Ag ¹²⁵ I ¹²⁵ Iodine labelled 25OH Vit D (HPLC grade).	1 vial 168 kBq lyophilised	red	Add 10.5 ml of Tracer Buffer
 CAL 0 Calibrator 0: in horse serum and phosphate buffer with gentamycin.	1 vial lyophilised	yellow	Add 0.5 ml distilled water
 CAL N Calibrators 1-5 in horse serum (see exact values on vial labels)	5 vials lyophilised	yellow	Add 0.5 ml distilled water
 DIL SPE Specimen diluent in horse serum	1 vial lyophilised	black	Add 1 ml distilled water
 WASH SOLN CONC Wash solution (TRIS-HCl)	1 vial 10 ml	brown	Dilute 70 x with distilled water (use a magnetic stirrer).
 CONTROL N Controls - N = 2 in human plasma with Proclin (see exact values on vial labels)	2 vials lyophilised	silver	Add 0.5 ml distilled water
 TRACER BUF Tracer Buffer with casein, gentamycin and red dye	1 vial 11.5 ml	red	Ready for use
 INC BUF Incubation Buffer with casein and proclin.	1 vial 55 ml	green	Ready for use

Note: Use Specimen diluent for dilution of samples with values above the highest calibrator before pre-treatment step.
No international reference material is available.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Distilled water
2. Pipettes for delivery of: 25 µl, 100 µl, 500 µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer

5. Tube shaker (300 to 700 rpm)
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system
8. Any gamma counter capable of measuring ¹²⁵I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

- A. Calibrators:** Reconstitute the calibrators with 0.5 ml distilled water.
- B. Controls:** Reconstitute the controls with 0.5 ml distilled water.
- C. Tracer:** Reconstitute the lyophilised tracer with 10.5 ml of the Tracer Buffer.
- D. Specimen diluent:** Reconstitute the lyophilised diluent with 1 ml distilled water.
- E. Working Wash solution:** Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators and controls are stable for one week at 2 to 8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 3 months. Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable, if kept in the original well-closed vial at 4°C for maximum one week or at -20°C (with one thawing) until the tracer expiry date.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- This kit is suitable for serum samples.
- Serum samples must be kept at 2-8°C.
- If the test is not run within 24 hrs, samples storage at -20°C is recommended.
- Avoid subsequent freeze-thaw cycles.

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiry date.
Do not mix materials from different kit lots.
Bring all the reagents to room temperature (18-25°C) prior to use.
Thoroughly mix all reagents and samples by gentle agitation or swirling.
Use a clean disposable pipette tip for addition of each different reagent and sample in order to avoid cross-contamination. High precision pipettes or automated pipetting equipment will improve the precision.
Prepare a calibration curve for each run, do not use data from previous runs.
Each tube can only be used once.

B. Procedure

The Incubation Buffer must be brought to room temperature (18-25°C) before beginning incubation.

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes.
2. Dispense 25 µl of calibrator or control or sample.
3. Dispense 500 µl of Incubation Buffer into each tube, except those for total counts.
4. Incubate for 2 hours at room temperature (18-25°C) on a tube shaker (300 to 700 rpm).

Be careful: don't aspirate and don't wash tubes before dispensing the tracer.

5. Dispense 100 µl of ¹²⁵Iodine labelled 25OH Vitamin D into each tube, including the uncoated tubes for total counts.
6. Shake the tube rack gently by hand to liberate any trapped air bubbles.
7. Incubate for 1 hour at room temperature (18-25°C) on a tube shaker (300 to 700 rpm).
8. Aspirate the content of each tube (except total counts). Be sure that the tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
9. Wash tubes with 2 ml Working Wash solution (except total counts) and aspirate. Avoid foaming during the addition of the Working Wash solution.

10. Wash tubes again with 2 ml Wash solution (except total counts) and aspirate.
11. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
12. Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula :

$$B/B0 (\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

3. Plot the (B/B0(%)) values for each calibrator point as a function of 25OH vitamin D concentration of each calibrator point. Reject obvious outliers.
4. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
5. By interpolation of the sample (B/B0 (%)) values, determine the total 25OH vitamin D concentrations of the samples from the calibration curve.
6. For each assay, the percentage of total tracer bound in the absence of unlabelled 25 OH vitamin D (B0/T) must be checked.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

25OH Vitamin D total	cpm	B/Bo (%)
Total count	67320	
Calibrator		
0.0 ng/ml	20520	100.0
5.8 ng/ml	16288	79.4
13 ng/ml	10274	50.0
35 ng/ml	6398	31.2
50 ng/ml	3926	19.1
100 ng/ml	1190	5.8

Note : 1 ng/ml = 2.5 pmol/ml

XIII. PERFORMANCE AND LIMITATIONS

A. Detection Limit

The LOB (Limit of Blank) was calculated by measuring the blank several times and was calculated as the mean + 1.65 standard deviations of the distribution of these values.

The LOB was calculated to be 0.8 ng/ml.

The LOD (limit of detection) was calculated as the LOB + 1.65 standard deviations of a low concentration sample tested in 10 different runs.

The LOD was calculated to be 1.9 ng/ml.

The LOQ (Limit of Quantitation) was calculated by testing 5 samples of low values 10 times. The LOQ was calculated to be 2.6 ng/ml.

B. Specificity

The percentage of cross reaction was determined by testing sera with spiked and unspiked cross reactants. The results are summarized in the following table:

Compound	Cross-Reactivity (%)
25OH-Vitamin D ₃	100
25OH-Vitamin D ₂	85
1,25(OH) ₂ -Vitamin.D ₃	4.1
1,25(OH) ₂ -Vitamin.D ₂	0.2
Vitamin D ₃	ND
Vitamin D ₂	0.1
3-epi-25 hydroxy Vitamin D ₃	0.4
24,25(OH) ₂ -Vitamin.D ₃	23
25,26(OH) ₂ -Vitamin D ₃	26.5

ND : Non detectable

The assay performance is not affected by hemolysis (5 g/L hemoglobin tested) and by bilirubinemia (1 g/L bilirubin tested). Bilirubin conjugate (1g/L tested), triglycerides (2 g/L tested) and ascorbic acid (Vitamin C) (1 g/L) don't interfere with this assay.

C. Precision

INTRA-ASSAY				INTER-ASSAY			
Sample	N	<X> ± SD (ng/ml)	C.V. (%)	Sample	N	<X> ± SD (ng/ml)	C.V. (%)
A	20	23.1 ± 1.1	4.7	A	12	21.0 ± 1.4	6.7
B	20	37.1 ± 1.7	4.7	B	12	36.6 ± 2.1	5.8

SD : Standard Deviation; CV: Coefficient of variation

D. Accuracy

RECOVERY TEST

Added 25OH-Vit.D ₃ (ng/ml)	Recovery (%)
25.4	82
14.3	81
7.8	104
Added 25OH-Vit.D ₂ (ng/ml)	Recovery (%)
13.8	92
9.0	85
4.2	81

DILUTION TEST

Sample dilution	Theoretical concent. (ng/ml)	Measured concent. (ng/ml)
1/1	95.1	95.1
1/2	47.6	43.1
1/4	23.8	24.3
1/1	61.8	61.8
1/2	30.9	31.7
1/4	15.4	14.0
1/1	76.8	76.8
1/2	38.4	37.9
1/4	19.2	17.9

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 20 and 30 minutes after the calibrator has been added to the coated tubes.

TIME DELAY

	0 minute (ng/ml)	20 minutes (ng/ml)	30 minutes (ng/ml)
Sample 1	12.2	8.9	9.7
Sample 2	27.9	31.6	28.6
Sample 3	44.2	45.6	45.3

XIV. INTERNAL QUALITY CONTROL

If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.

If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Do not freeze-thaw more than twice.

Acceptance criteria for the difference between the duplo results of the samples should rely on Good Laboratory Practises.

XV. 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: <10 ng/mL; Insufficiency: 10-29 ng/mL; Sufficiency: 30 to 100 ng/mL; Potential toxicity: >100 ng/mL.

XVI. PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only.

This kit contains ¹²⁵I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HbsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

For more information, see Material Safety Data Sheet (MSDS).

XVII. BIBLIOGRAPHY

- ZERWEKH J.E. (2008)
Blood biomarkers of Vitamin D status.
Am. J. Clin. Nutr., 87(suppl):1087S-91S.
- HOLICK M.F. (2006)
Resurrection of Vitamin D deficiency and rickets.
J. Clin. Invest., 116:2062-2072.
- HEANEY R.P. (2000)
Vitamin D: how much do we need, and how much is too much.
Osteoporos. Int., 11:553-555.
- DAWSON-HUGHES B., HEANEY R.P., HOLICK M.F., LIPS P., MEUNIER P.J. (1997)
Prevalence of Vitamin D insufficiency in an adult normal population.
Osteoporos. Int., 7:439-443.
- BISCHOFF-FERRARI H.A., GIOVANNUCCI E., WILLETT W.C., DIETRICH T., DAWSON-HUGHES B. (2006)
Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes.
Am. J. Clin. Nutr., 84:18-28.
- HOLICK M.F.(2004)
Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers and cardiovascular disease.
Am. J. Clin. Nutr., 80:1678S-1688S.

- HEANEY R.P. (2010)
Defining deficiency of vitamin D .
Clinical Laboratory International , October 2010, vol.34 : 16-19.
- HOLICK M.F. (2007)
Vitamin D deficiency.
N. Engl. J. Med., 357:266-281.
- TAHA N. M. , VIETH R.(2010)
The problem of an optimal target level for 25-Hydroxyvitamin D, the test for vitamin D nutritional status .
Clinical Laboratory International , November 2010, vol.34 : 28-30

XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS μ l	CALIBRATORS μ l	SAMPLE (S) CONTROLS μ l
INCUBATION (in coated tubes)			
Calibrators	-	25	-
Samples / controls	-	-	25
Incubation Buffer	-	500	500
Incubation	2 hours at RT (18-25°C) on a shaker (300 to 700 rpm) !Don't aspirate tubes		
Tracer	100	100	100
Incubation	1 hour at RT (18-25°C) on a shaker (300 to 700 rpm)		
Separation	-	Aspirate	
Working Wash solution	-	2.0 ml	
Separation	-	Aspirate	
Working Wash solution	-	2.0 ml	
	-	Aspirate	
Counting	Count tubes for 60 seconds		

Diasource's Instrumentation Service confirms that the kit is valid for use on the platform Stratec Riamat 300. If you need any additional information, please contact IVDInstrumentation@diasource.be

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