



Read entire protocol before use.

25-OH-Vitamin D3-Ria-CT

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles - Belgium

I. INTENDED USE

Immunoradiometric assay kit for the in vitro quantitative measurement of 25-OH-Vitamin D3 in human in serum and plasma.

II. GENERAL INFORMATION

A. Name: Bio-Line **25-OH-Vitamin D3-Ria-CT** Kit
B. Catalogue number : **BL-29-CT**: 100 tests
C. Manufactured by : Bio-Line S.A.
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II. APPLICATION AND INTENDED USE

A. Physiological function of 25OH-Vit.D3

25-Hydroxyvitamin D3 (25OHD3) is the trivial name of 9, 10-secocholesta-5, 7, 10(19)-triene-3 β , 25-diol. This secosteroid is produced in the liver by 25-hydroxylation of cholecalciferol or Vitamin D3. 25OHD3 is a precursor for other Vitamin D metabolites and has only a limited biological activity in itself. The most active derivative is 1 α ,25-Hydroxyvitamin D3, produced in the kidney (or placenta) by 1 α -hydroxylation of 25OHD3. This hormonally regulated steroid stimulates the intestinal absorption of both calcium and phosphorus. It also stimulates bone resorption and mineralisation thereby preventing the development of rickets or osteomalacia. This Vitamin D hormone might also be active in other tissues responsible for calcium transport (placenta, kidney, mammary gland, ...) and endocrine glands (beta-cells, parathyroid glands, ...). 25-hydroxyvitamin D3 is a main precursor for the metabolites.

B. Regulatory mechanism

The production of 25-hydroxyvitamin D mainly occurs in the liver although other tissues (intestine, kidney) might perform the same hydroxylation. Although there might be some feedback inhibition of 25-hydroxyvitamin D on its own production, a higher substrate availability (Vitamin D3) results in higher 25-hydroxyvitamin D production and higher circulating 25-hydroxyvitamin D concentrations in blood. No hormonal or other regulatory mechanism for its synthesis have been clearly identified.

C. Clinical applications

This assay is of importance for the diagnosis of Vitamin D deficiency or intoxication.

III. PRINCIPLES OF THE BIOSOURCE 25OH-Vit.D₃-RIA-CT

At first standards and samples (serum or plasma) are extracted with acetonitrile. A fixed amount of ¹²⁵I labelled 25OH Vitamin D₃ competes with the 25OH Vitamin D₃ from either extracted samples or standards for a fixed amount of specific antibody sites immobilized to the lower and inner surface of plastic tubes. After 2 hours incubation at room temperature, an aspiration step stops the competition reaction. The tubes are then washed with 3 ml washing solution and counted in a gamma counter.

V. REAGENTS PROVIDED

Reagents	100 tests Kit	Colour Code	Reconstitution		
Tubes coated with anti – 25OH-Vitamin D ₃	2 x 50	pink	Ready for use		
<table border="1" style="display: inline-table; vertical-align: middle;"> <tr> <td>Ab</td> <td>¹²⁵I</td> </tr> </table> ¹²⁵ I –25OH Vit D ₃	Ab	¹²⁵ I	1 vial lyophil. 240 kBq	red	Ready for use
Ab	¹²⁵ I				
<table border="1" style="display: inline-table; vertical-align: middle;"> <tr> <td>Zero</td> <td>stan</td> </tr> </table> Standard 0 (horse serum with preservative)	Zero	stan	1 vial lyophil.	yellow	Add 1 ml distilled water
Zero	stan				
<table border="1" style="display: inline-table; vertical-align: middle;"> <tr> <td>CAL</td> <td>N</td> </tr> </table> Calibrators 1-5 in horse serum with preservative (see exact value on vial labels)	CAL	N	5 vials lyophil.	yellow	Add 1.0 ml distilled water
CAL	N				
Incubation buffer	1 vial 45 ml	blue	Ready for use		
Acetonitrile	1 vial 25 ml	black	Ready for use		
Washing Buffer	1 vial 10 ml	brown	Dilute the contain in 700 ml distilled water (use a magnetic stirrer).		
<table border="1" style="display: inline-table; vertical-align: middle;"> <tr> <td>CONTROL</td> <td>N</td> </tr> </table> Controls 1 and 2 in human serum with preservative	CONTROL	N	2 vials lyophil.	silver	Add 1 ml distilled water
CONTROL	N				

Note: use Standard 0 for dilution of samples with values greater than the highest standard before extraction step.

VI. SUPPLIES NOT PROVIDED

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

1. Distilled water.
2. Ethanol absolute.
3. Pipettes for delivery of: 50 µl, 100 µl, 200 µl, 400 µl, 1 ml.
4. Glass tubes (12x75 mm) for extraction step.
5. Vortex mixer.
6. Centrifuge operating at 1500 g.
7. Magnetic stirrer.
8. 5 ml automatic syringe (Cornwall type) for washing.
9. Aspiration and washing device.
10. Gamma counter, set for I125 counting.

VII. REAGENT PREPARATION

A. Standards : Reconstitute the zero standard with 1 ml distilled water and other standards with 1 ml distilled water.

B. Controls : Reconstitute the controls with 1 ml distilled water.

C. I125 25OH-Vit.D₃ : Reconstitute with 6 ml of a mix of distilled

water/ethanol (50/50).

D. Wash solution : Dilute the content of the washing buffer in 700 ml distilled water, use a magnetic stirrer to homogenize.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

Unopened components: store the unopened kit at 2-8°C. The components

have their expiry date printed on the individual labels.

Reconstituted components and working solutions :

- The standards and controls have to be stored for maximum one week

at 4°C. For longer storage, they have to be frozen at -20°C.

- The reconstituted tracer has to be frozen after 1st use. Then, it is stable until the expiry date.

- Wash solution: store the working dilution at 2-8°C, maximum till the expiry date of the kit..

IX. SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma samples must be kept at 2-8°C.

- If the test is not run within 48 hours, storage at -20°C is recommended.

- After thawing serum or plasma samples should be mixed (Vortex), then centrifuged.

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiration date. Do not mix materials from different kit lots. Bring all the reagents to room temperature 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. Respect the incubation times.

Prepare a standard curve for each run, do not use data from previous runs.

B. Procedure

1. Extraction step :

1. Label glass tubes (12x75 mm) for extraction : 6 standards, 2 controls and up to 40 samples.

2. Add 0.5 ml acetonitrile to each tube.

3. Dispense 200 µl of each standard, control or sample in the respective tubes.

4. Mix for 7 seconds with a vortex.

5. Centrifuge for 5 minutes at room temperature (at 800 g).

2. Incubation step :

1. Label coated tubes, in duplicate, for each standard, control and sample.

For determination of total counts, label 2 normal tubes.

2. Add 100 µl of the supernatant obtained after the extraction step in the corresponding tubes. Pipette tips have to be saturated with corresponding supernatant before the addition in the tube .

3. Dispense 400 µl Incubation Buffer in each tube, except those for total counts.

4. Add 50 µl tracer in each tube, including total counts.

5. Shake the tube rack gently.

6. Incubate for 2 hours, at room temperature.

7. Aspirate the content of each tube (except total counts).

8. Wash tubes twice with 2 ml Wash Solution and aspirate. Avoid foaming during the addition of the wash solution.

After the washing, let the tubes standing upright for two minutes and aspirate the remaining drop of liquid

9. Count the tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations, rejecting obvious outliers.

2. Calculate the bound radioactivity as a percentage of the binding determined at the zero standard point (0) according to the following formula :

$$B/B_0(\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

- Using a 3 cycle semi-logarithmic or logit-log graph paper plot the (B/Bo x 100) values for each standard point as a function of the 25OH.D₃ concentration of each standard point. Computer assisted methods can also be used to construct the calibration curve.
- By interpolation of the sample (B/Bo x 100) values, determine the 25OH.D₃ concentration of the samples from the reference curve.
- For each assay, the percentage of total tracer bound in the absence of unlabelled 25OH.D₃ (Bo/T) must be checked.

XII. PERFORMANCE CRITERIA AND LIMITATIONS

A. Minimal detectable concentration

The M.D.C. is estimated at 0.6 ng/ml and is defined as the concentration of 25OH-Vitamin.D₃ which corresponds to a cpm level of 2 standard deviations below the mean of 20 replicates of the zero standard.

B. Specificity

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

Compound	Cross-Reactivity (%)
25OH-Vitamin.D ₂	0.6
1,25(OH) ₂ -Vitamin.D ₃	84.0*
24,25(OH) ₂ -Vitamin.D ₃	14
25,26(OH) ₂ -Vitamin.D ₃	8

* As 1,25(OH)₂-Vit.D₃ concentrations are practically 1000 times lower than 25-OH-Vit.D₃, this cross-reactivity is insignificant and does not interfere in this 25-OH-Vit.D₃ assay.

C. Precision

INTRA ASSAY				INTER ASSAY			
Serum	Replicate	<X> ± SD (ng/ml)	CV (%)	Serum	Replicate	<X> ± SD (ng/ml)	CV (%)
A	20	6.4 ± 0.5	7.9	A	13	20.7 ± 1.7	8.2
B	20	20.3 ± 1.2	6.1	B	13	52.8 ± 3.7	7.1

SD : Standard Deviation; CV: Coefficient of variation

D. Accuracy

RECOVERY TEST

Added 25OH-Vit D3 (ng/ml)	Mesured 25OH Vit D3 (ng/ml)	Recovery (%)
0	9.7	100
7.5	17.2	114
15	25.8	104
30	39.7	100
60	68.2	97.8

DILUTION TEST

Sample	Dilution	Theoretical Concent. (ng/ml)	Measured Concent. (ng/ml)
1	1/1	69.9	69.9
	1/2	34.9	35.0
	1/4	17.4	18.0
	1/8	8.7	9.3
	1/16	4.3	4.6
	1/32	2.1	1.9
	1/64	1.05	1.0
2	1/1	48.8	48.8
	1/2	24.4	23.4
	1/4	12.2	12.2
	1/8	6.1	5.5
	1/16	3.05	3.0
	1/32	1.52	1.58

XII. TYPICAL DATA

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

25-OH-Vitamin D3		cpm	B/T (%)
Total count		34870	B/Bo x 100
Calibrator	0.0 ng/ml	14490	100 %
	0.6 ng/ml	13199	91 %
	2.4 ng/ml	10948	76 %
	13.2 ng/ml	7703	53 %
	83 ng/ml	4375	30 %
	103 ng/ml	2584	18 %

Note : 1 ng/ml = 2.5 pmol/ml

XII. EXPECTED VALUES

Dietary intake, race, season and age are known to affect the normal levels of 25OH.Vit.D₃.

The normal values given hereafter should not be considered as absolute.

They were observed from a normal adult population (18-65 years) in Belgium. Samples were taken during the first seven months of the year 1996.

Vitamin D deficiency is usually seen when serum concentrations fall below 4 ng/ml and Vitamin D intoxication is present or imminent with values exceeding 150 ng/ml.

NORMAL ADULT SUBJECTS (n = 209)

Mean (ng/ml)	S.D. (ng/ml)	Range (ng/ml)	2.5 - 97.5 percentile (ng/ml)
36	15.4	7.6-75.0	11-70

XIII. PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only.

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 enduser's country. In no case the product must be administered to humans or animals.

The human blood components in this kit have been tested and found non reactive for HbsAg, anti HCV, anti HIV 1 and anti HIV 2. However no known method can assure the absence of infectious material such as hepatitis, aids, other infected blood components. Therefore the handling of the reagents and patient samples should be in accordance with the local safety procedures.

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 metalazides. 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. All radioactive handling should be executed in a designated area, away from regular passage. A log book 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 radiosafety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the notified bodies holding jurisdiction over the laboratory. Adherence to the basic rules of the radiation safety provides adequate protection.

XIII. BIBLIOGRAPHY

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XIX. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS ml	CALIBRATORS ml	SAMPLE(S) ml
Extraction			
Acetonitrile	-	0.5	0.5
Standards	-	0.2	-
Samples/ Controls	0.05	-	0.2
Vortex	Vortex for 7 secondes		
Centrifugation	5 minutes at 800 g		
Incubation			
Supernatant of extraction	-	0.1	0.1
Incubation Buffer	-	0.4	0.4
Tracer	0.05	0.05	0.05
Incubation	2 hours at Room Temperature		
Separation			
Wash Solution	-	Aspirate	
	-	2 ml	
Separation	-	Aspirate	
Wash Solution	-	2 ml	
Separation	-	Aspirate carefully	
Counting	Count tubes for 60 seconds		

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