IVD

CE

25OH-Vitamin D total-RIA-CT

KIP1971 - KIP1974

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B - 1348 Louvain-la-Neuve – Belgium

History

Summary of change:

<table>
<tr><th>Previous Version:
200430-1</th><th>Current Version:
200615</th></tr>
<tr><td>

V. REAGENTS PROVIDED

Reagents	96 Tests Kit	4x 96 Tests Kit	Colour Code	Reconstitution
[Ag|125I] 125Iodine labelled 25OH Vit D (HPLC grade).	1 vial 160 kBq lyophilised	4 vials 160 kBq lyophilised	red	Add 6 ml of Tracer Buffer
[TRACER|BUF] Tracer Buffer with casein, gentamycin and red dye	1 vial 7 ml	4 vials 7 ml	red	Ready for use

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V. REAGENTS PROVIDED

Reagents	96 Tests Kit	4x 96 Tests Kit	Colour Code	Reconstitution
[Ag|125I] 125Iodine labelled 25OH Vit D (HPLC grade).	1 vial 168 kBq lyophilised	4 vials 168 kBq lyophilised	red	Add 10.5 ml of Tracer Buffer
[TRACER|BUF] Tracer Buffer with casein, gentamycin and red dye	1 vial 11.5 ml	4 vials 11.5 ml	red	Ready for use

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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)

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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)

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<tr><td>

VII. REAGENT PREPARATION

C. Tracer: Reconstitute the lyophilised tracer with 6 ml of the Tracer Buffer.

</td><td>

VII. REAGENT PREPARATION

C. Tracer: Reconstitute the lyophilised tracer with 10.5 ml of the Tracer Buffer.

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X. PROCEDURE

5. Dispense 50 µl of ^{125}Iodine labelled 25OH Vitamin D into each tube, including the uncoated tubes for total counts.

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X. PROCEDURE

5. Dispense 100 µl of ^{125}Iodine labelled 25OH Vitamin D into each tube, including the uncoated tubes for total counts.

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XII. TYPICAL DATA

25OH Vitamin D total		cpm	B/Bo (%)
Total count		52033	
Calibrator	0.0 ng/ml	17721	100.0
	10 ng/ml	11022	62.2
	20 ng/ml	6826	38.5
	40 ng/ml	3446	19.4
	60 ng/ml	1469	8.3
	100 ng/ml	592	3.3

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XII. TYPICAL DATA

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

</td></tr>
</table>

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 D3	0.8
Vitamin D2	0.1
3-epi-25 hydroxy Vitamin D3	0.4
24,25$(OH)_2$-Vitamin.D_3	≥100
25,26(OH)2-Vitamin D3	≥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 D3	ND
Vitamin D2	0.1
3-epi-25 hydroxy Vitamin D3	0.4
24,25$(OH)_2$-Vitamin.D_3	23
25,26(OH)2-Vitamin D3	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 COUNTS µl	CALIBRATORS µl	SAMPLE (S) CONTROLS µl
Tracer	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 COUNTS µl	CALIBRATORS µl	SAMPLE (S) CONTROLS µl
Tracer	100	100	100

Room temperature (18-25°C)

Review and corrections in the Korean version

CE

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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.

II. GENERAL INFORMATION

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

B. Catalog number : KIP 1971 : 96 tests
KIP 1974 : 4 x 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

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

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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 D_3 and 25OH Vitamin D_2 from Vitamin D Binding Protein (DBP).
Then, without washing steps, a fixed amount of ^{125}I labelled 25OH Vitamin D is added in each tube to compete with the 25OH Vitamin D_3 and 25OH Vitamin D_2 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 (D_3 and D_2) concentrations of the samples are determined by dose interpolation from the calibration curve.

V. REAGENTS PROVIDED

Reagents	96 Tests Kit	4x 96 Tests Kit	Colour Code	Reconstitution
Tubes coated with Mab anti 25OH Vit D3 and D2	2 x 48	8 x 48	pink	**Ready** for use
Ag | **^{125}I** ^{125}Iodine labelled 25OH Vit D (HPLC grade).	1 vial 168 kBq lyophilised	4 vials 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	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	5 vials lyophilised	yellow	**Add** 0.5 ml distilled water
DIL | **SPE** Specimen diluent in horse serum	1 vial lyophilised	2 vials lyophilised	black	**Add** 1 ml distilled water
WASH | **SOLN** | **CONC** Wash solution (TRIS-HCl)	1 vial 10 ml	4 vials 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	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	4 vials 11.5 ml	red	**Ready** for use
INC | **BUF** Incubation Buffer with casein and proclin.	1 vial 55 ml	2 vials 110 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 ^{125}I 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 ^{125}Iodine 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 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

The percentage of cross reaction was determined by testing sera with spiked and unspiked crossreactants. 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 D3	ND
Vitamin D2	0.1
3-epi-25 hydroxy Vitamin D3	0.4
24,25$(OH)_2$-Vitamin.D_3	23
25,26(OH)2-Vitamin D3	26.5

ND : Not 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 ^{125}I (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

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XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS µl	CALIBRATORS µl	SAMPLE (S) CONTROLS µl
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 ml	
Separation		Aspirate	
Working Wash solution	-	2 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

Other translations of this Instruction for Use can be downloaded from our website: https://www.diasource-diagnostics.com/

DIAsource Catalogue Nr : KIP1971	Revision nr : 200615

Revision date : 15/06/2020

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Leer el protocolo completo antes de usar.

25OH Vitamin D total -RIA-CT

I. INSTRUCCIONES DE USO

Radioinmunoensayo para la determinación cuantitativa in vitro de la 25-hidroxivitamina D3 y D2 (25-OH-D3 y 25-OH-D2) en suero.

II. INFORMACIÓN GENERAL

A. Nombre: DIAsource 25OH- Vitamin D total -Ria-CT

B. Número de Catálogo: KIP1971 : 96 test
KIP1974 : 4 x 96 test

C. Fabricado por: DIAsource ImmunoAssays S.A.

Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

Para cuestiones técnicas e información sobre pedidos contactar:
Tel : +32 (0)10 84.99.00 Fax : +32 (0)10 84.99.90

III. INFORMACIÓN CLÍNICA

Vitamina D es el término genérico usado para designar a la vitamina D3 o colecalciferol y la Vitamina D2 o ergoclaciferol.

Los humanos producen vitamina D3 en forma natural cuando la piel está expuesta a los rayos ultravioleta del sol.
La vitamina D3 es metabolizada principalmente en el hígado produciendo 25-Hidroxivitamina D3 (25 OH D3) que es la forma principal de vitamina D circulando en el organismo.
25 OH D3 es la precursora para otros metabolitos de la vitamina D y tiene una actividad limitada por si sola. El derivado más activo es la 1,25-Hidroxivitamina D3, producida en el riñón (o placenta) por la 1α-hidroxilación de 25OHD3.
La 25OHVitamina D estimula la absorción intestinal del calcio y el fósforo y también la reabsorción y mineralización ósea.
La 25OH Vitamina D también puede estar activa en otros tejidos siendo responsable del transporte de calcio (placenta, riñón, glándula mamaria…) y glándula endocrina (glándula paratiroides, células beta…).

La Vitamina D3 y Vitamina D2 también están disponibles por ingestión a través de los alimentos o suplementos dietéticos.
Como la Vitamina D2 se metaboliza en forma similar a la vitamina D3, ambas contribuyen al estado general de la Vitamina D de un individuo.
Por esta razón es muy importante medir ambos tipos de 25 OH Vitamina D de la misma forma para un diagnóstico correcto de deficiencia, insuficiencia o intoxicación.
La deficiencia de vitamina D es un factor de riesgo importante en raquitismo, osteomalacia, osteoporosis senil, cáncer y el resultado del embarazo.
La medición de ambos tipos de 25 OH Vitaminas D también es necesario para determinar la causa de concentraciones anormales de calcio en el suero de pacientes.
Se ha demostrado que la intoxicación con Vitamina D puede causar daño en el riñón y tejidos.

IV. PRINCIPIOS DEL MÉTODO

Al inicio, los calibradores, controles y muestras (suero) se incuban con el tampón de incubación, directamente en tubos recubiertos durante 2 horas a temperatura ambiente (18-25°C), en un agitador, para liberar 25OH Vitamina D_3 y 25OH Vitamina D_2 de la proteína fijadora de la Vitamina D (DBP).
A continuación y antes de lavar, se añade una cantidad fija de 25OH Vitamina D marcada con ^{125}I en cada tubo para competir con la 25OH Vitamina D_3 y la 25OH Vitamina D_2 de las muestras controles o calibradores, por una cantidad fija de sitios de un anticuerpo monoclonal inmovilizado en la zona interna inferior de los tubos de plástico.
Después de 1 hora de incubación a temperatura ambiente (18-25°C) en un agitador de tubos, una etapa de aspiración termina la reacción de competencia. Luego los tubos se lavan dos veces y se aspiran nuevamente. Se dibuja una curva de calibración y el total de las concentraciones de las 25 OH Vitaminas D (D_3 y D_2) de las muestras se determinan por interpolación de dosis usando la curva de calibración.

V. REACTIVOS SUMINISTRADOS

Reactivos	Kit de 96 pruebas	Kit de 4x 96 pruebas	Código de color	Reconstitución
Tubos recubiertos con el anticuerpo monoclonal anti 25OH Vit D3 y D2	2 x 48	8 x 48	rosa	**Listo** para uso
Ag | **^{125}I** 25OH Vit D marcada con ^{125}Yodo (grado HPLC).	1 vial 168 kBq liofilizado	4 viales 168 kBq liofilizado	rojo	**Añadir** 10,5 ml del tampón Trazador
CAL | **0** Calibrador 0: en suero equino y tampón de fosfato con gentamicina	1 vial liofilizado	1 vial liofilizado	amarillo	**Añadir** 0,5 ml de agua destilada
CAL | **N** Calibradores 1-5 en suero equino **(ver el valor exacto en la etiqueta del vial)**	5 viales liofilizado	5 viales liofilizado	amarillo	**Añadir** 0,5 ml de agua destilada
DIL | **SPE** Diluyente de la muestra diluido en suero de caballo	1 vial liofilizado	2 viales liofilizado	negro	**Añadir** 1 ml de agua destilada
WASH | **SOLN** | **CONC** Solución de lavado (TRIS-HCl)	1 vial 10 ml	4 viales 10 ml	marrón	**Diluir** x70 con agua destilada (utilizar un agitador magnético)
CONTROL | **N** Controles - N = 2 en plasma humano con proclina **(ver el valor exacto en la etiqueta del vial)**	2 viales liofilizado	2 viales liofilizado	plata	**Añadir** 0,5 ml de agua destilada
TRACER | **BUF** Tampón trazador con caseína y gentamicina y tinción roja	1 vial 11,5 ml	4 viales 11,5 ml	rojo	**Listo** para uso
INC | **BUF** Tampón de incubación con caseína y proclina	1 vial 55 ml	2 viales 110 ml	verde	**Listo** para uso

Nota : Utilizar el diluyente de la muestra para diluir muestras con valores superiores al valor del calibrador más alto antes del paso de pre tratamiento.
No existe ninguna preparación de referencia internacional.

VI. MATERIAL NO SUMINISTRADO

El material mencionado a continuación es necesario y no está incluido en el kit
1. Agua destilada
2. Pipetas de 25 µl, 100 µl, 500 µl y 1 ml (se recomienda el uso de pipetas precisas con puntas plásticas)
3. Vortex
4. Agitador magnético
5. Agitador de tubos (300 a 700 rpm)
6. Jeringa automática 5 ml (tipo Cornwall) para el lavado
7. Sistema de aspiración (opcional)
8. Contador de radiaciones gamma para medir I^{125} (mínima eficiencia 70%)

VII. PREPARACIÓN DE REACTIVOS

A. Calibradores: reconstituir los calibradores con 0,5 ml de agua destilada.
B. Controles: Reconstituir los controles con 0,5 ml de agua destilada.
C. Trazador: Reconstituir el trazador liofilizado con 10,5 ml de Tampón trazador.
D. Diluyente de la muestra: Reconstituir el diluyente liofilizado con 1 ml de agua destilada.
E. Solución de lavado de trabajo: Preparar el volumen necesario de Solución de lavado de trabajo mezclando 69 partes de agua destilada por 1 parte de Solución de lavado (70x). Utilizar un agitador magnético para homogeneizar. Desechar la solución de lavado de trabajo no utilizada al final del día.

VIII. ALMACENAJE Y CADUCIDADES DE LOS REACTIVOS

- Antes de abrir o reconstituir todos los componentes de los kits son estables hasta la fecha de caducidad indicada en la etiqueta, si se guardan a 2-8ºC.
- Después de su reconstitución los calibradores y controles son estables durante una semana a 2-8ºC. Para periodos más largos, alicuotar y guardar a –20ºC por 3 meses máximo. Evitar congelar y descongelar sucesivamente.
- La solución de lavado de trabajo recién preparada solo debe utilizarse en el mismo día. Después del primer uso, el trazador es estable, si se guarda en el vial original debidamente cerrado a 4°C, máximo por una semana o a -20°C (con una descongelación) hasta la fecha de caducidad
- Después del primer uso, el trazador es estable hasta la fecha de caducidad, si se mantiene en el vial original, debidamente cerrado a -20°C (con una descongelación) o máximo durante una semana a 4°C.
- Las alteraciones de los reactivos en el aspecto físico pueden indicar inestabilidad o deterioro.

IX. RECOGIDA Y PREPARACIÓN DE MUESTRAS

- Este kit es adecuado para muestras de suero.
- Las muestras de suero deben ser guardadas a 2-8ºC.
- Si el ensayo no se realiza en 24 horas, almacenar las muestras a –20º.
- Evitar congelar y descongelar sucesivamente.

X. PROTOCOLO

A. Notas de manejo

No utilizar el kit o componentes después de la fecha de caducidad. No mezclar reactivos de diferente número de lote. Llevar todos los reactivos a temperatura ambiente (18-25°C) antes de su uso.
Agitar minuciosamente todos los reactivos y muestras, agitándolos o girándolos suavemente. Con el fin de evitar contaminación cruzada utilizar puntas de pipetas desechables y limpias para la adición de cada reactivo y muestra.
El uso de pipetas de precisión o equipamiento de dispensación automática mejorara la precisión. Respetar los tiempos de incubación.
Preparar la curva de calibración en cada ensayo, no utilizar los datos de un ensayo previo.
Cada tubo solo se puede usar una vez.

B. Procedimiento

El tampón de incubación debe alcanzar temperatura ambiente (18-25°C) antes de iniciar la incubación.

1. Etiquetar los tubos recubiertos en duplicado para cada calibrador, control y muestra. Para la determinación del conteo total, etiquetar 2 tubos normales.
2. Dispensar 25 µl de calibrador o control o muestra.
3. Dispensar 500 µl de tampón de incubación en cada tubo, excepto en los destinados a conteo total.

4. Incubar durante 2 horas a temperatura ambiente (18-25°C) en un agitador de tubos (300 a 700 rpm).

Precaución : no aspirar y no lavar los tubos antes de dispensar el trazador.

5. Dispensar 100 µl de 25OH Vitamina D marcada con ^{125}Yodo encada tubo incluyendo los tubos no recubiertos para conteo total.
6. Agitar la gradilla con tubos suavemente con la mano para liberar cualquier burbuja de aire atrapada.
7. Incubar durante 1 hora a temperatura ambiente (18-25°C) en un agitador de tubos (300 a 700 rpm).
8. Aspirar el contenido de cada tubo (excepto los de conteo total). Asegurar que la punta del aspirador alcance el fondo del tubo recubierto para sacar todo el líquido.
9. Lavar los tubos con 2 ml de solución de trabajo de lavado (excepto los de conteo total) y aspirar. Evitar la formación de espuma durante la adición de la solución de trabajo de lavado.
10. Lavar los tubos nuevamente con 2 ml de solución de lavado (excepto los conteos totales) y aspirar.
11. Dejar los tubos en la posición vertical durante dos minutos y aspirar la gota de líquido remanente.
12. Leer los tubos en un contador gamma durante 60 segundos.

XI. CALCULO DE RESULTADOS

1. Calcular la media de los duplicados.
2. Calcular la radiactividad enlazada como un porcentaje de la unión determinada al punto cero (0) del calibrador de acuerdo con la siguiente formula:

$$B/B0(\%) = \frac{\text{Cuentas (Calibrador o muestra)}}{\text{Cuentas(Calibrador Cero)}} x100$$

3. Representar los valores de (B/B0%) de cada punto del calibrador frente a las concentraciones de la 25OH vitamina D de cada calibrador, rechazando los puntos externos.
4. Métodos computarizados de resultados pueden ser utilizados para la construcción de la curva de calibración. Si se utiliza un sistema automático de cálculo de resultados, se recomienda usar la representación gráfica " 4 parámetros".
5. Por interpolación de los valores (B/B%) de las muestras, se determinan los valores de las concentraciones totales de la 25OH vitamina D desde la curva de calibración.
6. El porcentaje total de enlace del trazador en ausencia de la 25OH vitamina D no marcado (B0/T) debe ser calculado en cada ensayo.

XII. EJEMPLO DE RESULTADOS

Los datos mostrados a continuación sirven como ejemplo y nunca deberán ser usados como una calibración real.

25OH Vitamin D total		cpm	B/Bo (%)
	Cuentas Totales	67320	
Calibrador	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

Nota : 1 ng/ml = 2,5 pmol/ml

XIII. REALIZACIÓN Y LIMITACIONES

A. Límite de detección

El LOB (límite de blanco) se calculó midiendo el blanco varias veces y corresponde a la media + 1.65 desviación estándar de la distribución de estos valores. El LOB se calcula a 0,8 ng / ml.

El LOD (límite de detección) se calculó como la LOB + 1.65 desviación estándar de una muestra de baja concentración analizada en 10 ensayos diferentes. LOD se calcula en 1,9 ng / ml.

El límite de cuantificación (LOQ) se calculó analizando 5 muestras de valores bajos 10 veces. El límite de cuantificación se calculó en 2,6 ng / ml.

B. Especificidad

El porcentaje de reacción cruzada se determinó probando suero con y sin añadido de sustancias que producen reacción cruzada. Los resultados se han resumido en la siguiente tabla:

Compuesto	Reacción-cruzada (%)
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 D3	ND
Vitamin D2	0,1
3-epi-25 hidroxilo Vitamina D3	0,4
24,25$(OH)_2$-Vitamin.D_3	23
25,26(OH)2-Vitamina D3	26,5

ND : Indetectable

El rendimiento del ensayo no se ve afectado por hemólisis (probado con 5 g/l hemoglobina), por bilirrubinemia (probado con 1 g/l de bilirrubina). La bilirrubina conjugada (probado con 1g/l), los triglicéridos (probado con 2 g/l) y el ácido ascórbico (Vitamina C) (1 g/l) no interfieren con este ensayo.

C. Precisión

PRECISIÓN INTRA-ENSAYO / PRECISIÓN INTER-ENSAYO

Muestra	N	<X> ± SD (ng/ml)	CV (%)	Muestra	N	<X> ± SD (ng/ml)	CV (%)
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 : Desviación Estándar; CV: Coeficiente de Variación

D. Exactitud

TEST DE RECUPERACIÓN

25OH-Vit.D_3 añadido (ng/ml)	Recuperado (%)
25,4	82
14,3	81
7,8	104
25OH-Vit.D_2 añadido (ng/ml)	**Recuperado (%)**
13,8	92
9,0	85
4,2	81

TEST DILUCIÓN

Dilución de la muestra	Concent. Teórica (ng/ml)	Concent. Medida (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. Tiempo de espera entre la dispensación del último calibrador y la de la muestra

Como se muestra a continuación la precisión del ensayo se mantiene incluso en el caso de dispensar la muestra 20 y 30 minutos después de haberse adicionado el calibrador a los tubos recubiertos.

TIEMPO DE ESPERA			
	0 minutos (ng/ml)	20 minutos (ng/ml)	30 minutos (ng/ml)
Muestra 1	12,2	8,9	9,7
Muestra 2	27,9	31,6	28,6
Muestra 3	44,2	45,6	45,3

XIV. CONTROL DE CALIDAD INTERNO

Si los resultados obtenidos del Control 1 y /o el Control 2 no caen dentro del rango especificado en la etiqueta del vial, los resultados no se pueden utilizar a no ser que dé una explicación satisfactoria por la discrepancia.

Si así lo desean, cada laboratorio puede preparar sus propias mezclas de muestras de control, que deben guardarse congeladas en alíquotas. No congelar y descongelar más de dos veces.

El criterio de aceptación paralas diferencias entre los dos resultados de las muestras deben basarse en Buenas Prácticas de Laboratorio.

XV. VALORES ESPERADOS

La alimentación, la raza, la estación y la edad pueden influenciar los niveles normales de 25OH.Vitamin.D3.
Cada laboratorio debe establecer su propio rango basado en su población local.

Literatura reciente ha sugerido los siguientes rangos para la clasificación del estado de la 25 OH Vitamina D: Deficiencia: <10 ng/ml; Insuficiencia: 10-29 ng/ml; Satisfactorio: 30 a 100 ng/ml; Potencialmente tóxico: >100 ng/ml.

XVI. PRECAUCIONES Y ADVERTENCIAS

Seguridad

Para uso solo en diagnóstico in vitro.
Este kit contiene I^{125} (vida media : 60 días) emisor de rayos X (28 keV) y de rayos γ (35,5 keV) ionizantes.
Este producto radiactivo solo puede ser manejado y utilizado por personas autorizadas; la compra, almacenaje, uso y cambio de productos radiactivos están sujetos a la legislación del país del usuario. En ningún caso el producto deberá ser suministrado a humanos o animales.
Todo el manejo de producto radiactivo se hará en un área señalizada, diferente de la de paso regular. Deberá de utilizarse un libro de registros para recepción y almacenaje de productos radiactivos utilizados. El material de laboratorio, vidrio que podría estar contaminado radiactivamente deberá separarse para evitar la contaminación cruzada con otros radioisótopos.
Cualquier derramamiento radiactivo deberá ser limpiado de inmediato de acuerdo con los procedimientos de seguridad. Los desperdicios radiactivos deberán ser eliminados de acuerdo con las regulaciones y normativas vigentes de la legislación a la cual pertenezca el laboratorio. El ajustarse a las normas básicas de seguridad radiológica facilita una protección adecuada.
Los componentes de sangre humana utilizados en este kit han sido testados por métodos aprobados por la CEE y/o la FDA dando negativo para HBsAg, anti-HCV, anti-HIV-1 y 2. No se conoce ningún método que asegure que los derivados de la sangre humana no transmitan hepatitis, SIDA u otras infecciones. Por lo tanto el manejo de los reactivos y muestras se hará de acuerdo con los procedimientos de seguridad locales.
Todos los productos animales y derivados han sido obtenidos a partir de animales sanos. Componentes bovinos originales de países donde BSE no ha sido informado. Sin embargo, los componentes conteniendo substancias animales deberán ser considerados como potencialmente infecciosos.
Evitar cualquier contacto de los reactivos con la piel (azida sódica como conservante). La azida en este kit puede reaccionar con el plomo y cobre de las cañerías y producir azidas metálicas altamente explosivas. Durante el proceso de lavado, hacer circular mucha cantidad de agua por el sumidero para evitar el almacenamiento de la azida.
No fumar, beber, comer o utilizar cosméticos en el área de trabajo. No pipetear con la boca. Utilizar ropa de protección y guantes.
Para obtener más información, consulte la Hoja de datos de seguridad del material (MSDS).

XVII. BIBLIOGRAFÍA

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XVIII. RESUMEN DEL PROTOCOLO

	CONTEO TOTAL µl	**CALIBRADORES** µl	**CONTROLES DE LAS MUESTRAS** µl
INCUBACIÓN (en tubos recubiertos)			
Calibradores	-	25	-
Muestras / controles	-	-	25
Tampón de incubación	-	500	500
Incubación	2 horas a TA (18-25°C) en agitador (300 a 700 rpm) ***¡No aspirar los tubos!***		
Trazador	100	100	100
Incubación	1 hora a TA (18-25°C) en agitador (300 a 700 rpm)		
Separación	-	Aspirar	
Solución de trabajo de lavado		2 ml	
Separación		Aspirar	
Solución de trabajo de lavado	-	2 ml	
		Aspirar	
Conteo	Leer los tubos durante 60 segundos		

El servicio de instrumentación de DIAsource confirma que el kit es válido para su uso en la plataforma Stratec Riamat 300. Si necesita información adicional, comuníquese con IVDInstrumentation@diasource.be

DIAsource Catalogo Nr : KIP1971	Revisión nr : 200615

Fecha de la revisión : 15/06/2020

DIAsource 25OH-VIT D Total-RIA-CT [체외진단의료기기]

I. 제품개요

번호	항 목	내 용
1	품목명	골구성물질대사측정검사시약
2	제품명	DIAsource 25OH-VITD total-RIA-CT
3	허가번호	체외수인 15-269 호
4	사용목적	사람의 혈청 내 25OH VIT D2와 25OH VIT D3 정량 측정
5	포장단위	96 테스트/키트
6	저장방법	2-8℃, 제조일로부터 70일
7	사용기한	2-8℃, 제조일로부터 70일

II. 측정원리

먼저 Vitamin D Binding Protein (DBP)에서 25OH VITD3와 25OH VITD2를 분리하기 위해 표준용액과 정도관리용액, 검체(혈청)를 배양완충액과 함께 코팅된 시험관에서 실온에서 2시간동안 배양한다. 그런 후 세척 단계 없이 시험관 아래의 내부에 고착시킨 고정된 양의 특이 항체 부위에 대해 추출한 검체나 표준용액, 정도관리용액으로부터의 25OH VITD3와 25OH VITD2의 경합을 위해 ^{125}I 표지 25OH Vitamin D를 추가한다. 실온에서 1시간의 배양 후, 흡입 단계를 시행하여 경합반응을 중지시킨다. 그런 후 시험관은 2번 세척하고 Counter에서 계수된다.

III. 제공되는 시약

번호	명칭	구성	Color Code	재구성
1	Coated tubes	2 X 48	Pink	즉시 사용 가능
2	Tracer ^{125}I labeled 25OH-Vit D	1 vial, 동결건조 168 kBq	Red	Tracer Buffer 10.5ml 첨가
3	Calibrator 0	1 vial, 동결건조	Yellow	증류수 0.5ml 첨가
4	Calibrators 1 - 5	5 vials, 동결건조	Yellow	증류수 0.5ml 첨가
5	Specimen Diluent	1 vial, 동결건조	Black	증류수 1ml 첨가
8	Wash Solution Concentration	1 vial, 10ml	Brown	증류수로 70배 희석 (자력교반기 사용)
9	Control I & II	2 vials, 동결건조	Silver	증류수 0.5ml 첨가
6	Tracer Buffer	1 vial, 11.5ml	Red	즉시 사용 가능
5	Incubation Buffer	1 vial, 55ml	Green	즉시 사용 가능

Note: (전처리 단계 전) 가장 높은 값의 표준용액 보다 높은 값을 가진 검체의 희석에는 표본 희석제를 사용.

IV. 측정절차

1. 검체 준비
   (1) 혈청만 사용가능하며 2-8℃에 보관한다.
   (2) 측정이 24시간 이내에 이루어지지 않는다면 검체는 -20℃에 냉동 저장해야 한다.
   (3) 냉동/해동 반복을 피한다.
2. 시약 조제
   (1) 표준용액: 0.5ml의 증류수를 첨가하여 재구성한다.
   (2) 정도관리 용액: 0.5ml의 증류수를 첨가하여 재구성한다.
   (3) 트레이서: 트레이서 완충액 10.5ml를 첨가하여 재구성한다.
   (4) 검체 희석액: 1ml의 증류수를 첨가하여 재구성한다.
   (5) 세척용액: 적절한 양의 희석된 세척용액을 준비하기 위해 세척용액 대 증류수의 양을 69 대 1로 (70배 희석) 한다. 균질화하기 위해 자력교반기를 이용한다. 재구성한 세척용액은 사용 후 폐기한다.
3. 검사 방법 (*자동화 장비 : Gamma Pro)

Note 1: 배양완충액은 사용 전 실온(18-25° C)으로 가져와야 한다.

Note 2: 트레이서를 분주하기 전에는 시험관을 흡입하거나 세척하지 않는다.

(1) 각 calibrator, control, 그리고 검체를 위한 코팅된 투브를 2개씩 준비하여 라벨을 부착한다. Total count는 2개의 일반 시험관을 준비한다.
(2) Calibrator, control, 및 검체 25ul 준비하여 각 시험관에 분주한다.
(3) Total count를 제외한 각 시험관에 배양완충액 500ul를 차례대로 첨가한다.
(4) 실온(18-25℃)에 300-700 RPM에 설정된 shaker로 2시간 동안 반응시킨다.
(5) 반응이 끝난 후 total count를 포함한 모든 시험관에 100ul의 트레이서를 분주한다.
(6) 시험관 랙을 손으로 부드럽게 흔들어 모든 기포를 제거한다.
(7) 실온(18-25℃)에 300-700 RPM에 설정된 tube shaker로 1시간 동안 반응시킨다.
(8) Total count를 제외한 모든 시험관의 액체를 흡입하여 제거한다. 흡입기의 끝 부분이 바닥에 닿아서 모든 액체가 제거 될 수 있도록 한다.
(9) Total count를 제외한 모든 시험관을 2ml의 세척용액으로 세척한 후 액체를 흡입하여 제거한다. 세척용액을 첨가할 때 거품이 생기지 않도록 조심한다.
(10) ***(9)***번을 다시 반복한다.
(11) 세척 후 약 2분 동안 시험관을 똑바로 세워 놓은 후 남은 액체를 모두 흡입하여 제거한다.
(12) 60초 동안 Gamma Counter로 cpm을 측정한다.

4. 결과 산출
   (1) 자료정리
   ① 두 번 측정한 값의 평균값을 구한다.
   ② 아래의 공식을 이용하여 결합된 방사능을 계산한다.

$$B / B0 (\%) = \frac{\text{Counts (Calibrator 또는 검체)}}{\text{Counts (Zero Calibrator)}} \quad 0$$

   ③ 각 calibrator po H Vitamin D 농도의 함수로 표시한다. 이상치는 제외한다.
   ④ 자동으로 계산할 경우 4-Parameter Logistic Function 곡선적합(curve fitting)을 권장한다.
   ⑤ 각 검체의 25OH Vitamin D 농도는 보간법으로 표준곡선에서 산출한다.
   ⑥ 각 검사 마다 B0 / T (%)를 확인해야 한다.
   (2) 참고치
   결핍: < 10 ng/ml
   불충분: 10 - 29 ng/ml
   충분: 30 - 100 ng/ml
   독성가능: >100 ng/ml
5. 표준 데이터
   다음 자료는 예시일 뿐, 실제 표준곡선을 대신하여 사용해서는 안 된다.

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

V. 완제품 시험규격

1. 외관검사
   제조원의 품질관리표준지침서(문서번호 POCQ075)에 따라 시험하고, 확인양식(문서번호 FTPK004)에 기입하고 확인한다.
   (1) 문서번호 ITPKKIP1971에 기입된 대로 구성품이 일치하는지 확인한다
   (2) 제품 구성표의 lot와 키트안의 구성품이 일치하는지 확인
   (3) 구성품과 키트의 유효기간을 확인
   (4) 구성품의 라벨상태를 확인
   (5) 구성품의 포장상태를 확인(용량, 물질 등)
   (6) 서류가 맞게 있는지 확인(사용설명서, 품질서류 등)
   (7) 박스에 라벨이 정확히 부착되어 있는지 확인
   (8) 검사 후 담당자는 확인양식(FTPK004)에 기입하고 서명한다.

VI. 사용시 주의사항

1. 체외진단용으로만 사용하여야 하며, 체외진단용 이외 흡입이나 체내 투여 등을 금지한다.
2. 동 제품에 포함된 방사성동위원소 취급 시 다음 사항을 준수하여야 한다.
   (1) 방사성동위원소는 지정된 장소에 보관하며, 관련 법령에 따라 자격을 갖춘자가 지정된 장소에서 사용한다.
   (2) 방사성동위원소를 취급할 때 안전에 영향을 주는 불필요한 행동을 하지 않는다. (예, 음식 섭취, 흡연, 화장 등)
   (3) 방사성동위원소를 포함한 시약을 분주해야 하는 경우, 입으로 파이펫팅 하지 않는다.
   (4) 방사성동위원소를 취급할 때에는 장갑 및 실험복을 착용하며, 검사가 완료되면 손을 깨끗이 닦는다.

(5) 유출된 모든 물질은 즉시 닦아 낸 후 폐기 또는 취급에 관련된 소관 법령에 따라 처리하여야 하며, 방사성 물질의 오염이나 방사성 물질 등의 분실은 관련 법령에 정한 규정된 절차에 따라 처리한다.

3. 검사를 실시하기 전에 모든 제품(구성품 포함)은 해당 제품별 검사 온도 조건에 따라 실시한다.
4. 그밖에 방사성동위원소의 보관, 이동, 사용 및 폐기 등 취급에 관한 사항은 관련 법규 또는 규정에 따른다.
5. 본 kit 내의 혈액성분은 시험을 거쳤고 , HbsAg, 항HIV 1와 항 HIV 2에 대한 반응은 없었다. 알려져 있는 어떠한 방법으로도 간염 , AIDS, 감염성혈액 성분 같은 감염성 물질의 부재를 확신시킬 수 없다 그러므로 시약과 환자 검체의 취급은 병원내의 안전절차에 따라야 한다.
6. 시약이 피부에 접촉되지 않게 하라(요오드화나트륨 방부제). 본 kit 내의 요오드화합물 은 배관계통의 납과 구리와 반응하여 큰 폭발성을 가진 요오드화금속으로 변화할 수있다. 세척 단계에서 요오드화합물의 생성을 막기 위해 흐르는 물로 배수관을 씻어 내도록 한다.
7. 방사성물질의 취득과 저장에 대한 일지는 실험실 내에 보관되어야 한다. 방사성 물질로 오염될 수 있는 서로 다른 방사성물질에 의한 교차 오염을 예방하기 위해 실험실 기구와 유리제품은 서로 분리 되어져야 한다.
8. 방사성 물질이 쏟아진 경우에는 방사선안전 절차에 따라 즉시 제염하여야 한다. 방사성 폐기물은 특정 규정과 실험실의 관할권을 가지고 있는 신고당국의 지침에 따라 처리되어 야만 한다. 방사선안전에 대한 기본 규칙의 준수는 충분한 방호를 제공한다.