

1,25(OH)₂-Vitamin D RIA (CT) (RIA-4782)



Revised 10 Nov. 2011 rm (Vers. 5.1)

USA:

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

Radioimmunoassay for measurement of human 1,25(OH)₂-Vitamin D (1,25(OH)₂-Vit.D) in serum and plasma.

PRINCIPLES OF THE METHOD

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 a polystyrene 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.

REAGENTS PROVIDED

	Reagents	48 Test Kit	Colour Code	Reconstitution
TUBES	Tubes coated with anti 1,25(OH) ₂ -Vitamin D	1 x 48	green	Ready for use
Ag ¹²⁵J	Tracer: ¹²⁵ Iodine labelled 1,25(OH) ₂ -Vitamin D (HPLC grade) in phosphate buffer with bovine casein and gentamycin.	1 vial lyophilised 75 kBq	red	Add 26 mL reconstitution solution
CAL N	Calibrators - N = 1 to 5: Calibrators (see exact values on vial labels) in phosphate buffer with bovine casein and gentamycin	5 vials lyophilised	yellow	Add 2 mL elution solution
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 = 1 or 2: in human plasma with gentamycin	2 vials lyophilised	silver	Add 2 mL distilled water
REC SOLN	Reconstitution Solution: phosphate buffer with bovine casein and azide (<0.1%)	1 vial 30 mL	black	Ready for use
ELU SOLN	Elution Solution: phosphate buffer with bovine casein, methanol and azide (<0.1%)	1 vial 30 mL	green	Ready for use
GEL	Bond Elut Silica cartridges	20		Store at R.T.

Note : Use elution solution for calibrator 0 and for dilution of samples with values above the highest calibrator (dilute after separation step).

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SUPPLIES NOT PROVIDED

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

1. Distilled water
2. Diisopropylether (p.a.)
3. Cyclohexane (p.a.)
4. Ethyl acetate (p.a.)
5. Ethanol absolute (p.a.)
6. Dichloromethane (p.a.)
7. Pipettes for delivery of: 200 µL, 500 µL, 1 mL and 2 mL (the use of accurate pipettes with disposable plastic tips is recommended)
8. Glass tubes (12 x 75 mm) for extraction and for elution. (closed with a cap for the extraction step)
9. Glass tubes (16 x 100 mm) or (12 x 120 mm), or polypropylene tubes (falcon 2097), for the washing of the cartridges.
10. Vortex mixer
11. Magnetic stirrer
12. Centrifuge operating at 800 g.
13. Tube shaker (1200 rpm)
14. 5 mL automatic syringe (Cornwall type) for washing
15. Aspiration system (optional)
16. Any gamma counter capable of measuring ¹²⁵I may be used (minimal yield 70%).

REAGENT PREPARATION

- A. Calibrators:**
Reconstitute the calibrators with 2 mL elution solution (**just before the incubation step**).
- B. Controls:**
Reconstitute the controls with 2 mL distilled water.
- C. I¹²⁵ 1,25(OH)₂-Vitamin.D :**
Reconstitute with 26 mL of reconstitution solution.
- D. 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.
- E. Extraction solvent :**
2 mL for each control or sample to be tested, are needed. **Prepare a fresh solution** of diisopropylether, cyclohexane, ethyl acetate, (50, 40, 10 v/v).
- F. Washing solvent :**
1 mL for each control or sample to be tested, are needed.
Prepare a fresh solution of diisopropylether, cyclohexane, ethyl acetate, ethanol absolute (50, 40,10, 1 v/v).

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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; except the cartridges which must be stored at room temperature.
- The calibrators and controls are very unstable, use them immediately after reconstitution, freeze immediately in aliquots and keep them at –20°C for 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 until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Use freshly prepared extraction solvent and washing solvent, do not store them.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

SPECIMEN COLLECTION AND PREPARATION

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

If the test is not run within 24 hrs, storage in aliquots, at -20°C is recommended.

Avoid subsequent freeze-thaw cycles.

After thawing, the samples should be vortexed and centrifuged.

Serum or plasma (EDTA and heparin) provides similar results.

PROCEDURE

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 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 calibration curve for each run; do not use data from previous runs.

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Procedure

Extraction step : ! Only for controls and samples.

1. Label glass tubes (12x75 mm) for extraction: 2 controls and up to 16 samples.
2. Add 0.5 mL control or sample in the respective tubes.
3. Dispense 2 mL extraction solvent in each tube.
4. Tubes are closed with a cap and placed on a shaker for 1 hour at 1200 rpm.
5. Centrifuge each tube for 5 minutes at room temperature (at 800 g).
6. Supernatants are needed for the next step of separation.

Separation step : ! Only for controls and samples

1. Label glass tubes (12x75 mm) for extraction: 2 controls and up to 16 samples.
2. Put one "Bond Elut" cartridge in each tube.
3. Apply 1.6 mL of supernatant (2 x 0.8 mL), obtained after extraction step, on cartridge.
4. Then, wash cartridges with 1 mL washing solvent (cfr reagent preparation). ! Be careful never apply vacuum on cartridges, just let solvent draw by gravity.
5. Add 300 µL dichloromethane on each cartridge, let draw by gravity.
6. Add 300 µL of distilled water on each cartridge.
7. Centrifuge each tube for 5 minutes at room temperature (at 800 g).
8. Label glass tubes (12 x 75 mm) for elution of 1,25(OH)₂-Vitamin D. After centrifugation, transfer cartridges in the corresponding glass tubes.
9. Apply 400 µL elution solution on each cartridge to elute 1,25 (OH)₂-Vitamin D and centrifuge 5 minutes at room temperature (at 800 g).
10. **Vortex** the eluted fraction.

Note : After this step, samples must be incubated in coated tubes as soon as possible to avoid degradation.

Incubation step

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes
2. Briefly vortex calibrators (use elution solution as zero calibrator), extracted controls and samples and dispense 150 µL of each into the respective tubes.
3. Dispense 500 µL of ¹²⁵Iodine labelled 1,25(OH)₂-Vitamin D into each tube, including the uncoated tubes for total counts.
4. Shake the tube rack gently by hand to liberate any trapped air bubbles.
5. Incubate overnight at room temperature
6. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.

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7. Wash tubes with 2 mL Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
8. Aspirate (or decant) the content of each tube (except total counts).
9. Wash tubes again with 2 mL Wash solution (except total counts) and aspirate (or decant).
10. After the last washing, let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
11. Count tubes in a gamma counter for 60 seconds.

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 :

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

3. Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B₀(%)) values for each calibrator point as a function of the 1,25(OH)₂-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 1,25(OH)₂-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 1,25(OH)₂-Vitamin D (B₀/T) must be checked.

TYPICAL DATA

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

1,25(OH) ₂ -Vitamin D	cpm	B/B ₀ (%)
Total count	51237	
Calibrator 0.0 pg/mL	20425	100.0
4.7 pg/mL	17833	87.3
12.2 pg/mL	15921	77.9
60.4 pg/mL	10602	51.9
160.0 pg/mL	7150	35.0
411.0 pg/mL	4587	22.5

To the best of our knowledge, no international reference material exists for this parameter.

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INTERNAL QUALITY CONTROL

- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.

REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

The observed ranges are based on 2.5% to 97.5% percentiles.

Population	Range (pg/mL)	Mean	SD	n
Normal subjects	19.6 – 54.3	35.3	10.6	51

PRECAUTIONS AND WARNINGS

Safety

For research 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.

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SUMMARY OF THE PROTOCOL

	TOTAL COUNTS μL	CALIBRATORS μL	SAMPLE(S) / CONTROLS μL
<u>EXTRACTION</u>			
Calibrators	-	-	-
Samples / Controls	-	-	500
Extraction solvent	-	-	2000
Shaking	1 hour at 1200 rpm		
Centrifugation	5 minutes at 800 g		
<u>SEPARATION</u>			
Supernatant from extraction step	-	-	1600
<u>CARTRIDGE</u>			
Supernatant	1600 μL		
Washing Solvent	1000 μL		
Dichloromethane	300 μL		
Distilled water	300 μL		
Centrifugation	5 minutes at 800 g		
Elution solution	400 μL		
Centrifugation	5 minutes at 800 g		
	Vortex		
<u>INCUBATION</u>			
Calibrators	-	150	-
Extracted samples	-	-	150
Tracer	500	500	500
Incubation	Overnight at R.T.		
Separation	-	Aspirate (or decant)	
Washing Solution	-	2 mL	
Separation	-	Aspirate (or decant)	
Washing Solution	-	2 mL	
Separation	-	Aspirate (or decant)	
Counting	Count tubes for 60 seconds in a gamma counter.		

As of 10/31/11_rm