1,25-Dihydroxy-Vitamin D RIA (CT)

Immunoradiometric Assay (coated tube) for the quantitative determination of 1,25(OH)2-Vitamin D in human serum and plasma.

REF MG11015

Σ 48

2-8 °C

EU: IVD CE U.S.: For research use only. Not for use in diagnostic procedures.

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Read entire protocol before use.

**1,25-Dihydroxy-Vitamin D RIA (CT)**

**I. INTENDED USE**

Radioimmunoassay for the *in vitro* quantitative measurement of human 1,25(OH)$_2$-Vitamin D (1,25(OH)$_2$-Vit.D) in serum and plasma.

**II. GENERAL INFORMATION**

A. **Proprietary name**: 1,25-Dihydroxy-Vitamin D RIA (CT)

B. **Catalog number**: MG11015

**III. CLINICAL BACKGROUND**

A. **Biological activities**

Vitamin D3 is mainly synthesized in the skin from 7-dehydrocholesterol and is partially from dietary origin. In the liver, Vitamin D3 is hydroxylated on carbon 25 to produce the obligatory intermediate 25-OH-D3. 25-OH-D3 must be metabolized further before it can carry out the functions of Vitamin D on intestine, kidney and bone. This subsequent reaction takes place exclusively in the kidney in the non-pregnant mammal. Thus 25-OH-D3 is further hydroxylated in the 1a-position to produce 1a,25 dihydroxyvitamin D3 (1α,25-(OH)$_2$D3).

In addition to renal tissue, placenta of pregnant women and macrophage cells in case of sarcoidosis can also produce some amount of 1α,25-(OH)$_2$D3. 1α,25-(OH)$_2$D3 is the active form of Vitamin D with regard to the known functions whereas 25-OH-D3 and Vitamin D3 itself can be excluded as being physiologically functional. Furthermore since 1α,25-(OH)$_2$D3 is produced in the kidney and has some of its functions in the bone and intestine, it must be considered as a hormone. This hormone stimulates the intestinal absorption of both calcium and phosphorus. It also stimulates bone resorption and mineralization thereby preventing the development of rickets and osteomalacia.

1α,25-(OH)$_2$D3 might also be active in other tissues responsible for Calcium transport (placenta, kidney, mammary gland, ...) and endocrine glands such as parathyroid glands. 1α,25-(OH)$_2$D3 is rapidly metabolized and its lifetime is approximately 2-4 h in plasma. Its main metabolite is calcitroic acid, a C-23 carboxylic derivative essentially without any biological activity. In addition to this pathway, 1α,25-(OH)$_2$D3 undergoes 24-hydroxylation to produce 1,24,25-trihydroxy-Vitamin D3. This compound has less biological activity than its parent and this metabolism is considered as a minor pathway.

The levels of 1α,25-(OH)$_2$D3 in plasma or serum is 100 to 1000 less than that of 25-OH-D3. Due to its low concentrations and the presence of many similar metabolites, the measurement of 1α,25-(OH)$_2$D3 requires extraction and separation either by HPLC or by column chromatography.

B. **Clinical application**

The measurement of circulating 1α,25-(OH)$_2$D3 is indicated in several disorders affecting calcium metabolism such as: sarcoidosis, renal failure, hyper and hypo-parathyroidism, rickets, tumor-associated hypercalcemia, Vitamin-resistant dysfunction and treatment with anti-convulsive medication.
IV. 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.

V. REAGENTS PROVIDED

<table>
<thead>
<tr>
<th>Reagents</th>
<th>48 Test Kit</th>
<th>Colour Code</th>
<th>Reconstitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUBES</td>
<td>1 x 48</td>
<td>green</td>
<td>Ready for use</td>
</tr>
<tr>
<td>TRACER</td>
<td>1 vial</td>
<td>red</td>
<td>Add 26 ml rec.</td>
</tr>
<tr>
<td>LYO</td>
<td>5 vials</td>
<td>yellow</td>
<td>Add 2 ml elution solution</td>
</tr>
<tr>
<td>CAL N</td>
<td>1 vial 10 ml</td>
<td>brown</td>
<td>Dilute 70 x with distilled water (use a magnetic stirrer).</td>
</tr>
<tr>
<td>LYO</td>
<td>2 vials</td>
<td>silver</td>
<td>Add 2 ml distilled water</td>
</tr>
<tr>
<td>RECON</td>
<td>1 vial 30 ml</td>
<td>black</td>
<td>Ready for use</td>
</tr>
<tr>
<td>ELU</td>
<td>1 vial 30 ml</td>
<td>green</td>
<td>Ready for use</td>
</tr>
<tr>
<td>GEL</td>
<td>20</td>
<td></td>
<td>Store at R.T.</td>
</tr>
</tbody>
</table>

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

VI. 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 (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%).

VII. 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. 125I 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 (70%). 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 disopropylether, cyclohexane, ethyl acetate, (50, 40, 10 v/v). Use freshly prepared Working Wash solution should be used on the same day.
F. Washing solvent: 1 ml for each control or sample to be tested, are needed. Prepare a fresh solution of disopropylether, cyclohexane, ethyl acetate, ethanol absolute (50, 40, 10, 1 v/v).

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; except the calibrators 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.

IX. 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.
- Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.
- Use freshly prepared extraction solvent and washing solvent, do not store them.
- Serum or plasma (EDTA and heparin) provides similar results.

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 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.

B. 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 (16 x 100 mm) or (12 x 120 mm), or polypropylene tubes (falcon 2097), for washing cartridges: 2 controls and up to 16 samples.
2. Put one "Bond Elut" cartridge in each tube.
III. 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. A 4 cycle semi-logarithmic or logit-log graph paper, plot the
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). Be sure that the plastic 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 (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.

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:

\[
\frac{B/Bo(\%)}{100} = \frac{Counts \ (Calibrator \ or \ sample)}{Counts \ (Zero \ Calibrator)}
\]

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

<table>
<thead>
<tr>
<th>Added 1,25(OH)2-Vit.D (pg/ml)</th>
<th>Blanked 1,25(OH)2-Vit.D (pg/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>225</td>
<td>95.2%</td>
</tr>
<tr>
<td>25.0</td>
<td>46.3</td>
<td>95.0%</td>
</tr>
<tr>
<td>50.0</td>
<td>70.0</td>
<td>100.2%</td>
</tr>
</tbody>
</table>

Conversion Factor:
From pg/ml to pmol/L: \( \times 2.4 \)
From pmol/L to pg/ml: \( \times 0.42 \)

To the best of our knowledge, no international reference material exists for this parameter.
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.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.

XV. 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.

<table>
<thead>
<tr>
<th>Population</th>
<th>Range (pg/ml)</th>
<th>Mean</th>
<th>SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>19.6 – 54.3</td>
<td>35.3</td>
<td>10.6</td>
<td>51</td>
</tr>
</tbody>
</table>

XVI. PRECAUTIONS AND WARNINGS

Safety

For *in vitro* diagnostic use only.

This kit contains $^{251}$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. 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 potentially infectious.

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.

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

XVIII. SUMMARY OF THE PROTOCOL

<table>
<thead>
<tr>
<th>TOTAL COUNTS µl</th>
<th>CALIBRATORS µl</th>
<th>SAMPLE (S) CONTROLES µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Supernatant from extraction step</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>1 hour at 1200 rpm</td>
<td>5 minutes at 800 g</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>5 minutes at 800 g</td>
<td>400 µl</td>
</tr>
<tr>
<td>Elution solution</td>
<td>5 minutes at 800 g</td>
<td>500 µl</td>
</tr>
<tr>
<td>CARTRIDGE</td>
<td>1600 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Supernatant</td>
<td>300 µl</td>
<td>300 µl</td>
</tr>
<tr>
<td>Washing Solvent</td>
<td>1000 µl</td>
<td>300 µl</td>
</tr>
<tr>
<td>Distilled water</td>
<td>300 µl</td>
<td>300 µl</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>5 minutes at 800 g</td>
<td>500 µl</td>
</tr>
<tr>
<td>Elution solution</td>
<td>400 µl</td>
<td>500 µl</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>5 minutes at 800 g</td>
<td>500 µl</td>
</tr>
<tr>
<td>INCUBATION</td>
<td>-</td>
<td>150 µl</td>
</tr>
<tr>
<td>Extracted samples</td>
<td>-</td>
<td>150 µl</td>
</tr>
<tr>
<td>Tracer</td>
<td>500 µl</td>
<td>500 µl</td>
</tr>
<tr>
<td>Incubation</td>
<td>Overnight at R.T.</td>
<td>-</td>
</tr>
<tr>
<td>Separation</td>
<td>-</td>
<td>Aspirate (or decant)</td>
</tr>
<tr>
<td>Washing Solution</td>
<td>-</td>
<td>2 ml</td>
</tr>
<tr>
<td>Separation</td>
<td>-</td>
<td>Aspirate (or decant)</td>
</tr>
<tr>
<td>Washing Solution</td>
<td>-</td>
<td>2 ml</td>
</tr>
<tr>
<td>Separation</td>
<td>-</td>
<td>Aspirate (or decant)</td>
</tr>
<tr>
<td>Counting</td>
<td>Count tubes for 60 seconds in a gamma counter.</td>
<td></td>
</tr>
</tbody>
</table>

XVII. BIBLIOGRAPHY


Revision date : 2014-01-08 (V02)
Symbols / Symbole / Symbôles / Símbolos / Σύµβολα

<table>
<thead>
<tr>
<th>REF</th>
<th>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθµός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιµοποιείται από:</td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συµπύκνωµα</td>
</tr>
<tr>
<td>LYO</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Λυοφιλιασµένο</td>
</tr>
<tr>
<td></td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.</td>
</tr>
<tr>
<td></td>
<td>Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluación. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.</td>
</tr>
<tr>
<td></td>
<td>Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.</td>
</tr>
<tr>
<td></td>
<td>Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται µακριά από θερµότητα και άµεση επαφή µε το φως του ηλίου.</td>
</tr>
<tr>
<td></td>
<td>Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
</tr>
<tr>
<td></td>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
</tr>
<tr>
<td>C AUTION!</td>
<td>Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!</td>
</tr>
</tbody>
</table>

Symbols of the kit components see MATERIALS SUPPLIED.

Symbols der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.

Voir MATERIEL FOURNI pour les symbôles des composants du kit.

Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.

Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.

Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.

Για τα σύµβολα των συστατικών του κιτ συµβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Symbols Version 3.5 / 2012-01-20