


REF			SYSTEM
09038086190	09038086500	300	cobas e 402 cobas e 801

English

System information

Short name	ACN (application code number)
VITDT 3	10194

Intended use

Binding assay for the in vitro quantitative determination of total 25-hydroxyvitamin D in human serum and plasma. This assay is to be used as an aid in the assessment of vitamin D sufficiency.

The electrochemiluminescence binding assay is intended for use on **cobas e** immunoassay analyzers.

Summary

Vitamin D is a fat-soluble steroid hormone precursor that is mainly produced in the skin by exposure to sunlight. Vitamin D is biologically inert and must undergo two successive hydroxylations in the liver and kidney to become the biologically active 1,25-dihydroxyvitamin D.¹

The two most important forms of vitamin D are vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol). In contrast to vitamin D₃, the human body cannot produce vitamin D₂ which is taken up with fortified food or given by supplements. In blood vitamin D₃ and D₂ are bound to the vitamin D binding protein (VDBP) and transported to the liver where both are hydroxylated to form 25-hydroxyvitamin D. It is commonly agreed that 25-hydroxyvitamin D is the metabolite to determine the overall vitamin D status as it is the major storage form of vitamin D in the human body. This primary circulating form of vitamin D is biologically inactive with levels approximately 1000-fold greater than the circulating 1,25-dihydroxyvitamin D. The half-life of circulating 25-hydroxyvitamin D is 2-3 weeks.

Most of the 25-hydroxyvitamin D, measurable in blood circulation, is 25-hydroxyvitamin D₃ whereas 25-hydroxyvitamin D₂ reaches measurable levels only in patients taking vitamin D₂ supplements.^{2,3,4} Vitamin D₂ is considered to be less effective.⁵

The most abundant product of 25-hydroxyvitamin D catabolism by 24-hydroxylase (CYP24A1) is 24,25-dihydroxyvitamin D.⁶ It accounts for 2-20 % of the total circulating 25-hydroxyvitamin D, has a half-life of approximately 7 days and is present in blood circulation at concentrations of up to approximately 10 nmol/L.^{6,7,8}

Vitamin D is essential for bone health. In children, severe deficiency leads to bone-malformation, known as rickets. Milder degrees of insufficiency are believed to cause reduced efficiency in the utilization of dietary calcium.⁹ Vitamin D deficiency causes muscle weakness; in elderly, the risk of falling has been attributed to the effect of vitamin D on muscle function.¹⁰ Vitamin D deficiency is a common cause of secondary hyperparathyroidism.^{11,12} Elevations of parathyroid hormone levels, especially in elderly vitamin D deficient adults can result in osteomalacia, increased bone turnover, reduced bone mass and risk of bone fractures.¹³ Low 25-hydroxyvitamin D concentrations are also associated with lower bone mineral density.¹⁴ In conjunction with other clinical data, the results may be used as an aid in the assessment of bone metabolism.

So far, vitamin D has been shown to affect expression of over 200 different genes. Insufficiency has been linked to diabetes, different forms of cancer, cardiovascular disease, autoimmune diseases, respiratory diseases and innate immunity.²

The Elecsys Vitamin D total III assay employs a vitamin D binding protein labeled with a ruthenium complex^{a)} as capture protein to bind 25-hydroxyvitamin D₃ and 25-hydroxyvitamin D₂. Cross-reactivity to 24,25-dihydroxyvitamin D is blocked by a specific monoclonal antibody.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating the sample (9 µL) with pretreatment reagent 1 and 2, bound 25-hydroxyvitamin D is released from the VDBP.

- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled vitamin D binding protein, a complex between the 25-hydroxyvitamin D and the ruthenylated VDBP is formed. A specific unlabeled antibody binds to 24,25-dihydroxyvitamin D present in the sample and inhibits cross-reactivity to this vitamin D metabolite.
- 3rd incubation: After addition of streptavidin-coated microparticles and 25-hydroxyvitamin D labeled with biotin, unbound ruthenium labeled VDBPs become occupied. A complex consisting of the ruthenylated VDBP and the biotinylated 25-hydroxyvitamin D is formed and becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

Reagents - working solutions

The **cobas e** pack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as VITDT 3.

PT1 Pretreatment reagent 1, 1 bottle, 7.3 mL:
Dithiothreitol 1 g/L, pH 5.5.

PT2 Pretreatment reagent 2, 1 bottle, 6.3 mL:
Sodium hydroxide 57.5 g/L.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Vitamin D binding protein-Ru/(bpy)₃²⁺, 1 bottle, 18.8 mL:
Ruthenium labeled vitamin D binding protein 150 µg/L; bis-tris propane buffer 200 mmol/L; albumin (human) 25 g/L; pH 7.5; preservative.

R2 25-hydroxyvitamin D-biotin, 1 bottle, 15.8 mL:
Biotinylated 25-hydroxyvitamin D 20 µg/L; bis-tris propane buffer 200 mmol/L; pH 8.6; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:

Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:

Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:



Danger

H290



May be corrosive to metals.

H314

Causes severe skin burns and eye damage.

H317

May cause an allergic skin reaction.

Prevention:

Elecsys Vitamin D total III

- P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
- P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

Response:

- P301 + P330 + P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
- P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
- P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
- P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved by the FDA or that are in compliance with the legal rules applicable to placing in vitro diagnostic medical devices for human use on the market in the European Union.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.^{15,16}

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	11 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K₂-EDTA and K₃-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + coefficient of correlation ≥ 0.95 and within a bias $\leq \pm 15\%$ at the medical decision point (30 ng/mL).

Stable for 8 hours at 20-25 °C, 4 days at 2-8 °C, 24 weeks at -20 °C (± 5 °C).

Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary

tubes (sample collection systems), follow the instructions of the tube manufacturer.

Adapt preanalytics protocol if required.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 09038116190, CalSet Vitamin D total III, for 4 x 1.0 mL
- REF 09038124190, PreciControl Vitamin D total III, for 6 x 1.0 mL
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- cobas e** analyzer

Additional materials for **cobas e 402** and **cobas e 801** analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized using internal standards which are traceable to the ID-LC-MS/MS 25-hydroxyvitamin D RMP.^{17,18}

The ID-LC-MS/MS is traceable to the National Institute of Standards and Technology Standard Reference Material 2972.¹⁹

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 11 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Vitamin D total III.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or nmol/L).

Conversion factors: $\text{nmol/L} \times 0.40 = \text{ng/mL}$
 $\text{ng/mL} \times 2.50 = \text{nmol/L}$

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	$\leq 1129 \mu\text{mol/L}$ or $\leq 66 \text{ mg/dL}$
Hemoglobin	$\leq 0.373 \text{ mmol/L}$ or $\leq 600 \text{ mg/dL}$
Intralipid	$\leq 300 \text{ mg/dL}$
Biotin	$\leq 2456 \text{ nmol/L}$ or $\leq 600 \text{ ng/mL}$
Rheumatoid factors	$\leq 1200 \text{ IU/mL}$

Criterion: $\pm 2.5 \text{ ng/mL}$ of initial value for samples $\leq 20.0 \text{ ng/mL}$, within $\pm 10 \%$ of initial value for samples $> 20.0 \text{ ng/mL}$ to 50.0 ng/mL and within $\pm 15 \%$ of initial value for samples $> 50.0 \text{ ng/mL}$.

The assay result is not affected in samples with biotin concentrations up to 600 ng/mL (2456 nmol/L). Some studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day.²⁰ Concentrations up to 1160 ng/mL have been described after a single dose of 300 mg biotin used in controlled settings.²¹

If the biotin threshold for the assay is exceeded the result will have a positive bias (e.g. 11% at 930 ng/mL).

Pharmaceutical substances

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special drugs were tested. No interference with the assay was found.

Special drugs

Drug	Concentration tested mg/L
EinsAlpha (alfacalcidol)	0.0018
ZEMPLAR (paricalcitol)	0.0012
Rocaltrol (calcitriol)	0.0010

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

$3.00\text{--}120 \text{ ng/mL}$ or $7.50\text{--}300 \text{ nmol/L}$ (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as $< 3.00 \text{ ng/mL}$ ($< 7.50 \text{ nmol/L}$). Values above the measuring range are reported as $> 120 \text{ ng/mL}$ ($> 300 \text{ nmol/L}$) or up to 240 ng/mL (600 nmol/L) for 2-fold diluted samples.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 2.0 ng/mL (5.0 nmol/L)

Limit of Detection = 3.0 ng/mL (7.5 nmol/L)

Limit of Quantitation = 6.0 ng/mL (15.0 nmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from $n \geq 60$ measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of $\leq 20 \%$.

Dilution

Samples with 25-hydroxyvitamin D concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2. The concentration of the diluted sample must be $\geq 40 \text{ ng/mL}$ ($\geq 100 \text{ nmol/L}$).

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

Due to different standardizations between methods, result variation may arise.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Clinical assessment should be taken into consideration when interpreting results. It should be taken into consideration that differences in 25-hydroxyvitamin D levels may exist with respect to gender, age, season, geographical latitude and ethnic groups.^{22,23}

Currently there is no standard definition of the optimal vitamin D status. Most experts agree that vitamin D deficiency should be defined as 25-hydroxyvitamin D of $\leq 20 \text{ ng/mL}$ ($\leq 50 \text{ nmol/L}$).²² Vitamin D insufficiency is recognized as $21\text{--}29 \text{ ng/mL}$.²² Similarly, the US National Kidney Foundation considers levels $< 30 \text{ ng/mL}$ to be insufficient or deficient.²⁴ The preferred level for 25-hydroxyvitamin D by many experts is now recommended to be $\geq 30 \text{ ng/mL}$ ($\geq 75 \text{ nmol/L}$).^{22,23,25,26} Other clinical references may show different values.

A reference range study was conducted with samples from apparently healthy donors from the United States. Samples were collected from southern, middle and northern sites in summer and winter. There were approximately equal numbers of males and females, and approximately 30 % of the donors had dark complexion. The age range was 22 to 79 years.

The values given are for information only and may vary from other published data.

	Season					
	All (n = 463)		Summer (n = 245)		Winter (n = 218)	
Unit	ng/mL	nmol/L	ng/mL	nmol/L	ng/mL	nmol/L
Mean	26.6	66.5	29.2	73.1	23.6	59.1
Median	25.7	64.1	27.7	69.2	22.8	57.1
2.5 th percentile	10.2	25.4	12.5	31.3	9.38	23.5

Elecsys Vitamin D total III

	Season					
	All (n = 463)		Summer (n = 245)		Winter (n = 218)	
Unit	ng/mL	nmol/L	ng/mL	nmol/L	ng/mL	nmol/L
97.5 th percentile	49.4	123	52.4	131	44.1	110

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and cobas e 801 analyzers					
Sample	Mean		Repeatability		CV
			SD		
	ng/mL	nmol/L	ng/mL	nmol/L	
HS ^{b)} 1	7.88	19.7	0.498	1.25	6.3
HS 2	19.2	48.0	0.483	1.21	2.5
HS 3	29.3	73.3	0.864	2.16	3.0
HS 4	59.3	148	0.931	2.33	1.6
HS 5	112	280	1.42	3.55	1.3
PC ^{c)} Vitamin D total III 1	20.2	50.5	0.643	1.61	3.2
PC Vitamin D total III 2	38.2	95.5	0.790	1.98	2.1

b) HS = human serum

c) PC = PreciControl

cobas e 402 and cobas e 801 analyzers					
Sample	Mean		Intermediate precision		CV
			SD		
	ng/mL	nmol/L	ng/mL	nmol/L	
HS 1	7.88	19.7	0.698	1.75	8.9
HS 2	19.2	48.0	0.883	2.21	4.6
HS 3	29.3	73.3	1.14	2.85	3.9
HS 4	59.3	148	1.42	3.55	2.4
HS 5	112	280	1.73	4.33	1.5
PC Vitamin D total III 1	20.2	50.5	0.946	2.37	4.7
PC Vitamin D total III 2	38.2	95.5	1.10	2.75	2.9

Method comparison

a) A comparison of the Elecsys Vitamin D total III assay (y) using the CDC Verification Samples with concentrations assigned by the CDC Vitamin D Reference Laboratory by ID-LC-MS/MS (x) gave the following correlations (ng/mL):

Number of samples measured: 158

Deming^{27,28}

$$y = 0.915x + 0.734$$

$$r = 0.979$$

The sample concentrations were between 5.64 ng/mL (14.1 nmol/L) and 133 ng/mL (332.5 nmol/L).

b) A comparison of the Elecsys Vitamin D total III assay, [REF] 09038086190 (cobas e 402 analyzer; y) with the Elecsys Vitamin D total III assay,

[REF] 09038086190 (cobas e 801 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 157

Passing Bablok²⁹

$$y = 1.05x + 1.02$$

$$r = 0.996$$

The sample concentrations were between 5.85 ng/mL (14.6 nmol/L) and 101 ng/mL (253 nmol/L).

c) A comparison of the Elecsys Vitamin D total III assay, [REF] 09038086190 (cobas e 801 analyzer; y) with the Elecsys Vitamin D total III assay, [REF] 09038078190 (cobas e 601 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 157

Passing Bablok²⁹

$$y = 0.952x - 0.850$$

$$r = 0.992$$

The sample concentrations were between 8.68 ng/mL (21.7 nmol/L) and 109 ng/mL (273 nmol/L).

Analytical specificity

A study was performed based on guidance from CLSI EP07-A2 to evaluate the cross-reactivity of the assay with other vitamin D metabolites. Samples containing the cross-reactants were prepared at three 25-hydroxyvitamin D concentrations (approximately 25, 40 and 60 ng/mL). The % cross-reactivity was calculated for each sample using the equation below and normalized to the cross-reactivity of 25-hydroxyvitamin D₃.³⁰

$$\% \text{ cross-reactivity} = \frac{(\text{mean conc. of spiked sample} - \text{mean conc. of unspiked sample})}{\text{spiked concentration}} \times 100 \%$$

The mean results from this study are summarized in the following table:

Cross-reactant	Concentration added ng/mL	Mean cross-reactivity %
25-hydroxyvitamin D ₃	50	100
25-hydroxyvitamin D ₂	50	105.0
24,25-dihydroxyvitamin D ₃	100	8.1
3-epi-25-hydroxyvitamin D ₃	50	122.4
3-epi-25-hydroxyvitamin D ₂	50	103.6
1,25-dihydroxyvitamin D ₃	100	n. d. ^{d)}
1,25-dihydroxyvitamin D ₂	100	0.4
Vitamin D ₃	1000	0.8
Vitamin D ₂	1000	0.7

d) n. d. = not detectable

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog. Roche.com for definition of symbols used):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume for reconstitution
	Global Trade Item Number



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Additions, deletions or changes are indicated by a change bar in the margin.

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