25-OH Vitamina D AUTO

25-OH Vitamin D AUTO

Anvisa 80115310260

ORDER INFORMATION

Cat. No.	Kit size
4270075K	R1 3x20mL + R2 1x15mL
4270075M	R1 3x20mL + R2 1x15mL
4270169.6R	R1 4x34,6mL + R2 4x10,3mL
4270250K	R1 5x40mL + R2 1x50mL
4270050K	R1 2x20mL + R2 1x10mL
4270025K	R1 1x20mL + R2 1x5mL
42700050K	R1 2x20mL + R2 2x5mL

INTENDED USE

The assay is intended for use in clinical laboratories for the quantitative determination of 25-OH vitamin D (vitamin D) in human serum and plasma, using automated chemistry analyzers. Measurement of vitamin D is used for the assessment of the vitamin D sufficiency.

For in vitro diagnostic use only.

SUMMARY [1-5]

Vitamin D is a steroid hormone involved in the active intestinal absorption of calcium and in the regulation of its homeostasis. Vitamin D has two forms: Vitamin D2 and Vitamin D3. Vitamin D2 is obtained from dairy products whereas Vitamin D3 is produced in the skin after exposure to ultraviolet light. In the liver, Vitamin D is hydroxylated at its carbon 25 to form 25-OH Vitamin D. This metabolite is the predominant circulating form of Vitamin D and is considered to be an accurate indicator of the general Vitamin D status of an individual. Vitamin D deficiency has been linked to many diseases including osteoporosis, rickets, and osteomalacia. Both dietary supplements of Vitamin D that are currently available in the market (Vitamin D2 and Vitamin D3) are converted to 25-OH Vitamin D in the liver. The sum of the concentrations of 25-OH Vitamin D2 and 25-OH Vitamin D3, in serum or plasma, is referred to as “Total 25-OH Vitamin D”. Accurate monitoring of total 25-OH Vitamin D level is critical in clinical settings.

METHOD

Immunoturbidimetric assay with latex particles.

PRINCIPLE

The 25-OH Vitamina D Auto Assay is a direct latex-enhanced immuno-turbidimetric assay. The assay’s proprietary reagents are designed to dissociate vitamin D from vitamin D binding proteins, found in serum or plasma specimens, while particles coated with anti-vitamin D antibodies bind to the dissociated vitamin D, thereby causing agglutination. This agglutination is detected as an absorbance change (700 nm), with the magnitude of the change being proportional to the quantity of total vitamin D in the sample. Specimen concentrations are determined by interpolation from a 5-point calibration curve prepared from calibrators of known concentrations.

REAGENTS

Components and composition

R1: Phosphate buffer solution (< 100 mM), 0.1% sodium azide.

R2: Suspension of latex particles (< 0.5%) coated with anti-vitamin D antibodies, ready to use.

STORAGE AND STABILITY

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C, protected from light and if contamination is avoided. Do not mix different lots of reagents.

Do not freeze the reagents!

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. The reagent contain sodium azide (< 0.1%) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
4. For diagnostic purposes, the results should always be assessed with the patient’s medical history, clinical examinations and other findings.
5. Do not use de reagent after the expiration date labeled on the outer box.
6. Assay calibration frequency is dependent on instrument used. Additionally, the assay should be recalibrated and controls run with each new lot of reagents.
7. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures.
8. For professional use only.

WASTE MANAGEMENT

Follow the requirements of the current guidelines about technical regulation for the management of healthcare service waste, as well as other equivalent biosafety practices.

REAGENT PREPARATION

The reagents are liquid stable, ready-to-use reagents.
Mix by inverting at least 10 times before use.

MATERIALS REQUIRED, BUT NOT PROVIDED

1. NaCl solution 9 g/L.
2. General laboratory equipment.

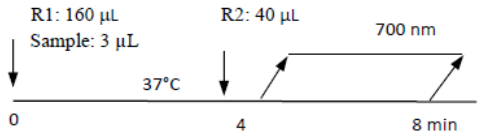
SPECIMEN

Serum, K2-EDTA plasma, K3-EDTA plasma or **Li-heparin plasma** samples can be used for the assay. Method comparison of K2-EDTA plasma samples versus serum samples yielded a regression equation of y = 1.0198x -0.4985 and R2 = 0.996. Method comparison of K3- EDTA plasma samples versus serum samples yielded a regression equation of y = 1.0378x – 1.2959 and R2 = 0.9944. Method comparison of Li-heparin plasma samples versus serum samples yielded a regression equation of y = 1.0475x – 1.3749 and R2 = 0.9947.
For plasma, mix the sample by gentle inversion prior to centrifugation. Centrifuge and separate serum or plasma as soon as possible after collection. The specimens may be refrigerated at 2-8°C for up to one week. For long term storage, they can be stored at -20°C or below. Avoid repeated freeze-thaw cycles (up to three cycles are acceptable). Do not use highly turbid or highly hemolyzed serum or plasma samples. Allow the refrigerated or frozen-thawed samples to equilibrate to room temperature for 30 minutes before use; samples must be mixed well before analysis.

ASSAY PROCEDURE

Applications for automatic systems are available upon request or on our website: www.kovalent.com.br

*General example of the assay test scheme and the specific application parameters for the Beckman AU 680 analyzer.



CALIBRATORS AND CONTROLS

For calibration in automated photometric systems, Kovalent TopKal 25-OH Vitamina D Auto calibrator is recommended. Use Kovalent Topkon 25-OH Vitamina D Auto (2 níveis) for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

WARRANTY

These instructions for use should be read carefully before using the product and the information contained therein should be strictly adhered to. The reliability of the test results cannot be guaranteed if the instructions are not followed.

RESULTS

Results are expressed in ng/mL.
Note: Samples with values greater than 147.8 ng/mL should be reported as >147.8 ng/mL. Samples with values less than 7.6 ng/mL should be reported as <7.6 ng/mL.

REFERENCE VALUES

Following C28-A3 Approved Guideline-Third Edition, reference range of the Vitamin D assay was determined by measuring the 25-OH vitamin D serum concentrations of a USA population of 145 apparently healthy adults, 21-67 years old, during the months of April and May (spring season). Individuals were from three different geographical locations: 47 from Pennsylvania (Northern US), 49 from Tennessee (Central US) and 49 from Texas (Southern US). All 145 individuals did not have kidney disease, GI disease, liver disease, calcium-levels related disease, thyroid disease, parathyroid disease, seizures, chronic disease or bariatric surgery. The central 95% of the population was found to have 25-OH vitamin D concentrations ranging between 7.2 and 41.6 ng/mL, with a mean concentration of 20.1 ng/ml.

LIMITATIONS

1. The assay is designed for use with human serum and plasma samples only.
2. As with any diagnostic test it is possible that technical, procedural errors as well as substances and factors not listed may interfere with the proper functioning of the test kit.
3. Heterophilic antibodies in human serum can react with reagent immunoglobulins or other reagent material, interfering with in vitro immunoassays. Patients routinely exposed to animals, animal serum

products, or other immunogenic products that may elicit heterophilic antibody production against the assay's reagents can be prone to this interference and anomalous values may be obtained. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions in an adult population.

PERFORMANCE CHARACTERISTICS

The following performance data was obtained on the Beckman AU680 chemistry analyzer.

Sensitivity / Limit of Detection

The LOB, LOD, and LOQ of the assay were determined following CLSI EP17-A2 guideline.

Limit of Blank LoB

Vitamin D-depleted serum was assayed with the Kovalent Vitamin D assay in five independent (over 5 days) runs with 12 replicates per run. The LoB was calculated as the mean of the 57th and 58th highest values for the blanks. The LoB of the assay was 1.2 ng/mL.

Limit of Detection LoD

Five very low vitamin D serum samples were measured in four independent runs (over 4 days), with 3 replicates per run. The LoD was defined as LoD = LoB + (1.645 * Standard Deviation of Low samples). The LoD of the assay was 2.9 ng/mL.

Limit of Quantitation LoQ

Five low Vitamin D samples were measured in 40 replicates obtained from five independent runs (5 days). The LoQ was measured as the lowest concentration with a CV of 20%. The claimed LoQ of the assay was 7.6 ng/mL.

Method comparison

The method comparison of the assay was evaluated following CLSI EP9-A3 guideline. A total of 171 serum samples were tested in comparison with a legally marketed 25-OH Vitamin D enzyme immunoassay.
The results for 171 serum samples are shown in the table below:

Deming Regression Analysis	95% Confidence Interval
Slope	1.062 (1.028 a 1.095)
Intercept	-3.03 (-4.94 a -1.11)
Correlation Coefficient	0.9785 (0.970 a 0.9841)
Range (ng/mL)	8.4 a 146.8

Precision

Precision was evaluated according to the CLSI EP5-A2 guideline. Controls and samples were measured daily over the span of 20 days, using three lots of reagents and one chemistry analyzer. 40 independent runs were performed on each specimen. Each run produced two measurements. 80 data points were obtained per specimen. Results are shown below:

25-OH Vitamina D (ng/mL)			Within-run		Between-run		Total	
Specimen	**n**	**Mean**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Control #1	80	21.7	0.9	3.9%	0.6	2.8%	1.3	6.2%
Control #2	80	42.5	1.0	2.4%	0.8	2.0%	1.7	3.9%
Sample #1	80	11.1	0.9	8.3%	0.5	4.4%	1.8	16.6%
Sample #2	80	18.2	0.9	4.9%	0.7	3.9%	1.6	8.7%
Sample #3	80	22.1	0.8	3.8%	0.8	3.8%	1.2	5.6%
Sample #4	80	42.8	0.9	2.0%	1.0	2.4%	1.3	3.1%
Sample #5	80	59.5	1.0	1.7%	0.7	1.2%	1.6	2.7%
Sample #6	80	80.2	1.3	1.6%	1.1	1.4%	2.0	2.5%
Sample #7	80	99.5	1.8	1.8%	1.5	1.6%	2.7	2.8%
Sample #8	80	117.6	2.2	1.9%	2.0	1.7%	3.7	3.2%
Sample #9	80	139.2	2.7	1.9%	2.6	1.8%	4.1	2.9%

Linearity

Eleven levels of linearity were prepared by diluting a high serum sample with vitamin D-depleted serum. Linearity levels were prepared according to the CLSI EP6-A guideline. Measurements were done in triplicates. The assay was found to be linear between 7.6 and 147.8 ng/mL.

Specificity / Interferences

Interference studies were conducted according to the CLSI EP7-A2 guideline. The acceptance criterion was set at 10% or less deviation between the spiked sample and the control. The assay's results were not significantly affected by the following endogenous substances:

Substância	Tolerância	Unidade
Free bilirubin	40	mg/dL
Conjugated bilirubin	40	mg/dL
Hemoglobin	600	mg/dL
Total protein	12.0	g/dL
Triglycerides	1000	mg/dL
Rheumatoid Factor (RF)	200	IU/mL

The assay's results were also not significantly affected by the following exogenous substances:

Substance	Tolerance	Unit
Acetaminophen	20.0	mg/dL
Acetyl Salicylic Acid	60.0	mg/dL
Ampicillin	5.3	mg/dL
Ascorbate	3.0	mg/dL
Biotin	100.0	ng/mL
Carbamazepine	3.0	mg/dL
Cefotaxime	180.0	mg/dL
Chloramphenicol	5.0	mg/dL
Creatinine	30.0	mg/dL
Digoxin	6.1	ng/mL
Ethanol	400.0	mg/dL
Ethosuximide	25.0	mg/dL
Furosemide	6.0	mg/dL
HAMA	350	ng/mL
Heparin	3.0	U/mL
Ibuprofen	50.0	mg/dL
Lidocaine	1.2	mg/dL
Lithium Acetate	2.2	mg/dL
Noradrenalin	4.0	ug/mL
Rifampicin	5.0	mg/dL
Theophylline	4.0	mg/dL
Urea	300.0	mg/dL
Uric Acid	20.0	mg/dL
Valproid Acid	50.0	mg/dL
Vancomycin	10.0	mg/dL

Cross-reactivity of the kit was determined by adding various vitamin D metabolites to serum pool samples. Based on the results in the table below, the assay did not cross-react with vitamin D2 or vitamin D3. The assay recovered 25-OH vitamin D2 and 25-OH vitamin D3 similarly. Cross-reactivity results are summarized in the table below:

Compound	Concentration tested	Cross-reactivity*
25-OH Vitamin D3	100.0 ng/mL	100%
25-OH Vitamin D2	100.0 ng/mL	106.9%
Vitamin D3	100.0 ng/mL	-0.8%
Vitamin D2	100.0 ng/mL	-1.7%
1,25-(OH)2 Vitamin D3	580.0 pg/mL	0.2%
1,25-(OH)2 Vitamin D2	580.0 pg/mL	-0.5%
24R,25-(OH)2 Vitamin D3	100.0 ng/mL	118.8%
3-epi-25-OH Vitamin D3	100.0 ng/mL	33.0%
3-epi-25-OH Vitamin D2	100.0 ng/mL	36.5%

*% Cross-reactivity = (Corrected Assay Value /Concentration Spiked)*100

Paricalcitol (Zemplar®) did not significantly cross-react with the assay when present at 5 ng/mL.

LITERATURE

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CONSUMER INFORMATION

Symbols used:

	Manufacturer
	Temperature limit
	In vitro diagnostic device
	Caution
	Operating instructions
	Recycling material
	Do not discard directly into the environment
	Batch code
	Date of manufacture
	Use by date
	Biological hazards
	Highly toxic
	Corrosive
	Harmful

Manufacturer:
Kovalent do Brasil Ltda.
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