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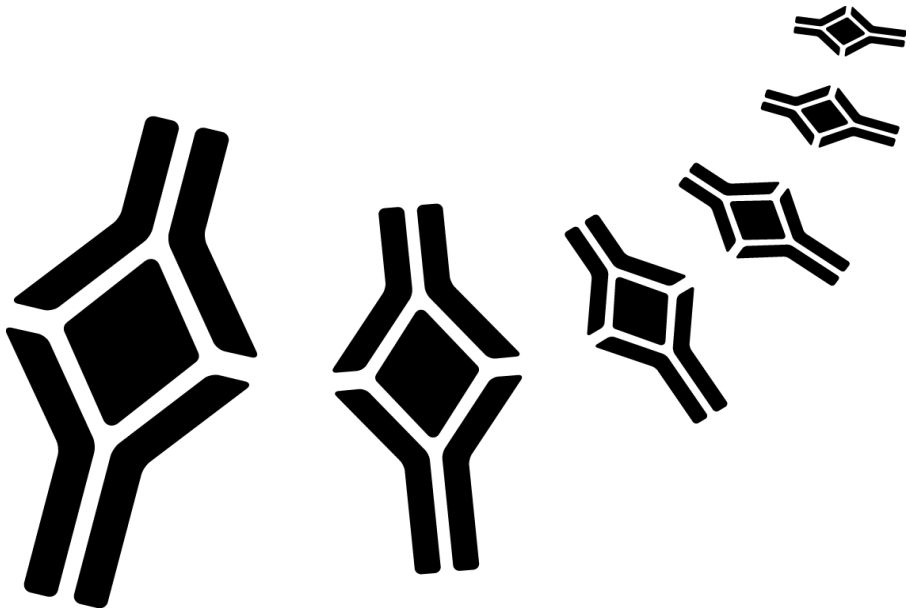

RAT 25OH VITAMIN D TOTAL ELISA

Product Data Sheet

Cat. No.: RIS022R

For Research Use Only

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» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RIS022R Rat 25OH Vitamin D Total ELISA is immunoenzymetric assay for the quantitative measurement of 25-hydroxyvitamin D2 and D3 (25OH-D2 and 25OH-D3) in rat serum.
It is intended for research use only.

2. STORAGE, EXPIRATION

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 eight 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.
Alterations in physical appearance of kit reagents may indicate instability or deterioration.

3. INTRODUCTION

Vitamin D is the generic term used to designate Vitamin D2 or ergocalciferol and Vitamin D3 or cholecalciferol. In the liver mainly, Vitamin D3 is metabolised into 25-Hydroxyvitamin D3 (25OH D3) which is the main form of Vitamin D circulating in the body. 25OH D3 is a precursor for other Vitamin D metabolites and has also a limited activity by itself.(1)
The most active derivative is 1,25-hydroxyvitamin D3, produced in the kidney (or placenta) by 1-hydroxylation of 25OH D3. 25OH Vitamin D stimulates the intestinal absorption of both calcium and phosphorus and also bone resorption and mineralisation. Rats have been used as experimental model to investigate the effect of 25OH Vitamin D supplements on fracture healing. (2)
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…).(3) 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.(4-5).
Studies on rats have been conducted to compare metabolism of oral Vitamin D2 and oral Vitamin D3.(6) and to define the effect of Vitamin D3 intake on calcium metabolism (7). Influence of vitamin D deficiency during intra uterine and early life on blood pressure has been suited in a rat model (8)
In studies on rat PTH (parathyroid hormone), 25OH vitamin D is measured in rat serum to determine its influence on PTH secretion.(9)

4. TEST PRINCIPLE

The Biovendor Rat 25OH Vitamin D Total ELISA is a solid phase Enzyme Linked Immunosorbent Assay performed on microtiterplates. During a first 2 hours incubation step, at room temperature, total 25OH Vitamin D (D2 and D3) present in calibrators, controls and samples is dissociated from binding serum proteins to fix on binding sites of a specific monoclonal antibody. After 1 washing step, a fixed amount of 25OH Vitamin D-labelled with biotin in presence of horseradish peroxidise (HRP), compete with unlabelled 25OH Vitamin D2 and 25OH Vitamin D3 present on the binding sites of the specific monoclonal antibody. After a 30 minutes incubation at room temperature, the microtiterplate is washed to stop the competition reaction. The Chromogenic solution (TMB) is added and incubated for 15 minutes. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is inversely proportional to the total 25OH Vitamin D (D2 and D3) concentration. A calibration curve is plotted and the total 25OH Vitamin D (D2 and D3) concentrations of the samples are determined by dose interpolation from the calibration curve.

5. PRECAUTIONS

Safety
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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 all reagents, Stop Solution contains HCl. In case of contact, wash thoroughly with water.
Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

6. TECHNICAL HINTS

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.
Perform calibrators, controls and samples in duplicate. Vertical alignment is recommended.
Use a clean plastic container to prepare the Wash Solution.
In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.

For the dispensing of the Chromogenic Solution and the Stop Solution avoid pipettes with metal parts.
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.
Dispense the Chromogenic Solution within 15 minutes following the washing of the microtiterplate.
During incubation with Chromogenic Solution, avoid direct sunlight on the microtiterplate.

7. REAGENT SUPPLIED

Reagents	**96 tests Kit**	**Color Code**	**Reconstitution**
Microtiterplate (96 breakable wells) with anti 25OHvit D2 and D3(MAbs)	96 wells		**Ready** for use
HRP | CONC Concentrate HRP	1 vial 0.2 ml	yellow	**Dilute** 200 x with conjugate buffer
CON | BUF Conjugate buffer with casein and proclin	1 vial 30 ml	red	**Ready** for use
CONJ | CONC 25OH Vitamin D Concentrated Conjugate	1 vial 0.3 ml	blue	**Dilute** 100 x with conjugate buffer
INC | BUF Incubation Buffer with casein and proclin	1 vial 20 ml	green	**Ready** for use
CAL | 0 Calibrator 0: biological matrix with gentamycin and proclin	1 vials lyophilized	yellow	**Add** 2.0 ml distilled water

CAL | N Calibrator N = 1 to 5 In horse serum with gentamycin and proclin	5 vials lyophilized	yellow	**Add** 1.0 ml distilled water
CONTROL | N Controls - N = 1 or 2 in rat serum with proclin	2 vials lyophilized	silver	**Add** 0.5 ml distilled water
WASH | SOLN | CONC Wash Solution (Tris-HCl)	1 vial 10 ml	brown	**Dilute** 200 x with distilled water (use a magnetic stirrer).
CHROM | TMB Chromogen TMB (Tetramethylbenzydine)	1 vial 12 ml	brown	**Ready** for use
STOP | SOLN Stop Solution: HCl 1.5N	1 vial 12 ml		**Ready** for use

Note: 1. Use the Calibrator 0 for dilution of samples with values above the highest calibrator
2. No international reference material is available.

8. MATERIAL REQUIRED BUT NOT SUPPLIED

1. High quality distilled water
2. Pipettes for delivery of: 50 µl, 150 µl, 200 µl, 1 ml, (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Plate shaker (400 rpm)
6. Washer for microtiterplates
7. Microtiterplate reader capable of reading at 450 nm and 650 nm (bichromatic reading)

9. PREPARATION OF REAGENTS

» Calibrators: Reconstitute the calibrator 0 with 1 ml distilled water and other calibrators with 1 ml distilled water.

» Controls : Reconstitute the controls with 0.5 ml distilled water.

» Working HRP Conjugate Solution:

!The working HRP conjugate solution is to be prepared during the incubation and minimum 1h45 minutes before its use. cf X.B.5)!

Prepare an adequate volume of working HRP conjugate solution by mixing concentrated conjugate, concentrated HRP and conjugate buffer according to the number of used strips, as indicated in the below table: for example for 6 strips (48 wells): 100µl of concentrated conjugate and 50 µl of concentrated HRP to 10 ml of conjugate buffer.

Use a vortex to homogenize.

Keep the working HRP conjugate at room temperature and avoid direct sunlight or use a brown glass vial for its preparation.

Number of stribs	Volume of Concentrated Conjugate (µl)	Volume of Concentrated HRP (µl)	Volume of Conjugate Buffer (ml)
1	30	15	3
2	50	25	5
3	60	30	6
4	80	40	8
5	90	45	9
6	100	50	10
7	120	60	12
8	140	70	14
9	160	80	16
10	180	90	18
11	200	100	20
12	220	110	22

» Working Wash solution : Prepare an adequate volume of Working Wash solution by adding 199 volumes of distilled water to 1 volume of Wash Solution (200x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

10. PREPARATION OF SAMPLES

This kit is suitable for rat serum samples only.
Serum samples must be kept at 2-8°C.
If the test is not run within 24 hrs, **sampling and storage at -20°C is recommended.**
Avoid subsequent freeze-thaw cycles.

11. ASSAY PROCEDURE

1. Select the required number of strips for the run. The unused strips should be resealed in the bag with a desiccant and stored at 2-8°C.
2. Secure the strips into the holding frame.
3. Pipette 50 µl of each Calibrator ,Controls and Sample into the appropriate wells.
4. Pipette 150 µl of Incubation Buffer into all the wells.
5. Incubate for 2 hours at room temperature, on a plate shaker (400 rpm)
   Prepare the Working HRP conjugate solution during the incubation and minimum 1h 45 minutes before its use.
6. Aspirate the liquid from each well.
7. Wash the plate 3 times by:
   - dispensing 0.35 ml of Wash Solution into each well
   - aspirating the content of each well
8. Pipette 200 µl of the working HRP conjugate solution into each well
   Incubate the microtiterplate for 30 minutes at room temperature, on a plate shaker (400 rpm)
9. Aspirate the liquid from each well.
10. Wash the plate 3 times by:
    - dispensing 0.35 ml of Wash Solution into each well
    - aspirating the content of each well
11. Pipette 100 µl of the Chromogenic solution into each well within 15 minutes following the washing step.
12. Incubate the microtiterplate for 15 minutes at room temperature, on a plate shaker (400 rpm), avoid direct sunlight.
13. Pipette 100 µl of Stop Solution into each well.
14. Read the absorbances at 450 nm (reference filter 630 nm or or 650 nm) within 1 hour and calculate the results as described in section XI

12. CALCULATIONS

Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
Calculate the mean of duplicate determinations.
Calculate for each calibrator, control and sample:

$$B/B0(\%) = \frac{OD\ (Calibrator, Control\ or\ Sample)}{OD\ (Zero\ Calibrator)} * 100$$

Using either linear-linear of semi-logarithmic graph paper, plot the
(B/B0(%)) values for each calibrator point as a function of the 25OH Vitamin D concentration of each calibrator point. Reject obvious outliers.
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.
By interpolation of the sample (B/B0 (%)) values, determine the 25OH Vitamin D concentrations of the samples from the calibration curve

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

25OD VitaminD ELISA		OD units
Calibrator	0 ng/ml	2.66
	5.3 ng/ml	2.39
	15 ng/ml	1.83
	25.7 ng/ml	1.46
	54.3 ng/ml	0.81
	133.0 ng/ml	0.21

Note: 1 ng/ml = 2.5 pmol/ml

13. PERFORMANCE CHARACTERISTICS

- **Detection Limit**

The Limit of Blank (LoB), Limit of Detection (LoD), and the Limit of Quantitation (LoQ), were determined in accordance with the CLSI guideline EP17-A.

The LoB was calculated by measuring the blank several times and calculating the 95th percentile of the distribution of the test values. The LoB was calculated to be 1.69ng/ml.
The LoD was calculated as described in the guideline. The LoD was calculated to be 2.81ng/ml.

The LoQ was calculated by testing 5 samples of low value 14 times in different test.
The LoQ was calculated to be 4.39ng/ml with CV of 20%.

- **Precision**

INTRA ASSAY				INTER ASSAY			
Sample	**N**	**<X> ± SD (ng/ml)**	**CV %**	**Sample**	**N**	**<X> ± SD (ng/ml)**	**CV %**
A	24	5.5±0.4	7.8	A	39	17.7±1.3	7.4
B	35	27.4±1.6	5.7	B	10	26.3±1.2	4.7
C	35	43.0±1.2	2.7	C	10	42.1±1.8	4.3
D	24	81.2±2.0	2.5	D	21	85.4±7.8	9.2

SD : Standard Deviation; CV: Coefficient of variation

- **Accuracy**

Dilution Test on a rat serum sample

Sample Dilution	Threoretical concetration (ng/ml)	Measured concetration (ng/ml)	Recovery (%)
1/1	39.4		
1/2	19.7	24	121
1/4	9.8	9.7	98.2
1/8	4.9	5.7	115

14. 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. Controls which contain azide will interfere with the enzymatic reaction and cannot be used.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises
- It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

15. REFERENCE INTERVALS

30 adult rats have been divided in 3 groups of 10 rats .
The first group has been fed with classical pellets for rodents containing 1200 UI 25OH vitamin D3 /kg and maintained in normal luminosity conditions (day and night)
The second group has been fed with pellets containing 3600 UI 25OH vitamin D3/kg and maintained in normal luminosity conditions (day and night).
The third group has been fed with pellets without 25 OH vitamin D3 and maintained in the dark day and night .
After 2 months , rat sera have been collected and tested : results in the table below.

	Group1 N=10	Group 2 N=10	Group 2 N=10
Mean valuet (ng/ml)	19.7	27.7	7.6
Range of values (ng/ml) (2.5 to 97.5 percentiles)	12.1-27.0	12.6-41.4	6.6-9.1

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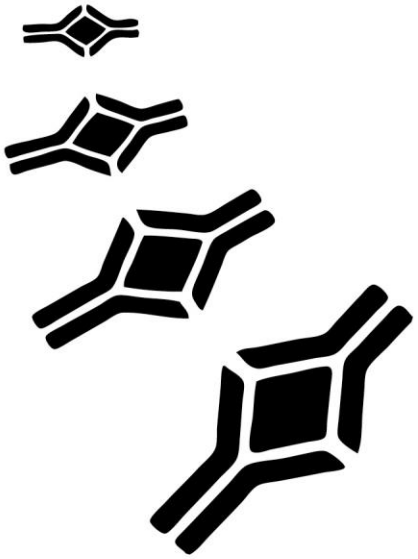
Eneral and comparative endocrinology 168: 312-317

» For more references on this product see our WebPages at www.biovendor.com

Assay procedure summary

	CALIBRATORS (µl)	SAMPLE(S) CONTROLS (µl)
Calibrators (0-6) Samples, Controls Incubation Buffer	50 - 150	- 50 150
Incubate for 2 hours at room temperature with continuous shaking at 400 rpm. Prepare the working HRP conjugate during the incubation and minimum 1h45minutes before its use Aspirate the contents of each well. Wash 3 times with 350µl of Wash Solution and aspirate.		
Working HRP Conjugate	200	200
Incubate for 30 minutes at room temperature with continuous shaking at 400 rpm. Aspirate the contents of each well. Wash 3 times with 350 µl of Wash Solution and aspirate.		
Chromogenic Solution	100	100
Incubate for 15 min at room temperature with continuous shaking at 400 rpm.		
Stop Solution	100	100
Read on a microtiterplate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm)		



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