DRG® 25 OH Vitamin D direct ELISA (EIA-4696)

REVISED 9 MAY 2011 (VERS. 10.1)

RUO IN THE USA

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

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

The ELISA is intended for determination of the 25-OH-Vitamin D in serum and fresh plasma.

2 MATERIAL SUPPLIED

CONTENT	KIT COMPONENTS	QUANTITY
PLATE	ONE HOLDER WITH PRECOATED STRIPS	12 X 8 WELLS
WASHBUF	ELISA WASH CONCENTRATE 20X	50 ML
RECSOL	RECONSTITUTION SOLUTION	2 X 20 ML
RELREAG	RELEASING REAGENT	2 X 1 VIAL
AB	ANTI 25-OH-VITAMIN D ANTIBODY, READY TO USE	18 ML
STD	STANDARDS, READY TO USE	6 VIALS, 300 µL EACH
CTRL	CONTROLS, READY FOR USE, (SEE SPECIFICATION FOR RANGE)	2 VIALS, 300 µL EACH
CONJ	CONJUGATE, EROXIDISE LABELED, READY TO USE	22 ML
SUB	TMB SUBSTRATE (TETRAMETHYLBENZIDINE)	2 X 15 ML
SAMDIL	SAMPLE DILUTION BUFFER	60 ML
STOP	ELISA STOP SOLUTION, READY TO USE	1 X 15 ML
FOL	FOIL TO COVER THE MICROTITER PLATE	2 X 1

3 MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water (aqua bidest.)
- Deep freezer -20 °C
- Precision pipettors calibrated to deliver 10-1000 µL
- Horizontal microtiter plate shaker
- Multi-channel dispenser or repeating dispenser
- Vortex-Mixer
- Water bath or heating block
- Standard laboratory glass or plastic vials, cups, etc. (one time products) made of polypropylene
- Microtiter plate reader 450 nm (reference wave length 620 or 690 nm)
- Refrigerator with **defined 8–10 °C**

4 PREPARATION AND STORAGE OF REAGENTS

- The test kit is designed for 96 single determinations. A reduction of the sample or buffer volumes results in erroneous values.
- To run assay more than once, ensure that reagents are stored at conditions stated on the label. The kit can be used up to 2 times within the expiry date stated on the label.
- The ***WASHBUF*** (wash buffer concentrate) should be diluted with aqua bidest. **1:20** before use (50 mL WASHBUF + 950 mL aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C in a water bath before dilution of the buffer solutions.
  The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label.
  Diluted buffer solution can be stored in a closed flask at **2-8°C for two weeks.**
- **Pre-heat *RECSOL (reconstitution solution)* for at least 20 min at 37°C in a water bath before use.**
- Reconstitute ***RELREAG (releasing reagent)*** in **16 mL pre-heated *RECSOL (reconstitution solution)***, mix gently by carefully swinging (do not vortex).
  The reconstituted RELREAG can be directly added into the prepared sample or kept at 37°C in a water bath until use.
  After use, aliquot, freeze remaining releasing reagent and store at -20 °C. Frozen RELREAG can be defrosted and used only once.
  Pre-heat frozen releasing reagent to 37° C before use (e.g. incubate for **at least 20 minutes at 37° C in a water bath**). Subsequently, it can be used right away.
- Bring ***AB*** (antibody) at room temperature at least one hour before use.

- All other test reagents are ready for use. The test reagents are stable up to the date of expiry (see label of test package) when stored at **2 –8 °C.**

  Note:

  1. Unused strips are covered and stored at 2 –8 °C. The covered strips should be used within 4 weeks.

  2. The remaining RELREAG is stored at -20 °C.

5 SPECIMEN COLLECTION AND PREPARATION

1. Fresh collected blood should be centrifuged within one hour. Vitamin D is an inert substance. However, serum storage at 2-8°C is recommended when the analysis is performed within 24 h after collection. Otherwise, the serum samples must be stored at -20°C until analyzed. **Avoid** repeated freeze-thaw cycles.
2. Serum samples can be shipped at 4-8 °C (for example with Coolpacks) and remain stable for up to 3 days.
3. **Serum** is the preferred sample matrix; whole blood is not suitable.
4. Indicated incubation times and temperatures must be strictly observed.
5. Mix samples well before use.

6 ASSAY PROCEDURE

6.1 Principle of the Test

The assay utilizes of a competitive ELISA technique with a selected monoclonal antibody recognizing 25(OH)-vitamin D. For a reliable determination of 25(OH)-Vitamin D, it is necessary to release it from the 25(OH)-vitamin D-DBP-complex.

Standards, controls and samples which are assayed for 25(OH)-vitamin D are incubated with the releasing reagent. The pre-incubated solutions are then transferred to the microplate coated with 25(OH)-vitamin D, and an anti-25(OH)-vitamin D antibody is added. During an over night incubation step, 25(OH)-vitamin D in the sample and a fixed amount of 25(OH)-vitamin D bound to the microtiter well compete for the binding of the antibody. Then a peroxidase-conjugated antibody is added into each microplate well. A complex of 25(OH)-vitamin D - anti-25(OH) vitamin D antibody – peroxidase conjugate is formed. Tetramethylbenzidine (TMB) is used as a peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction, whereby the color changes from blue to yellow.

The intensity of the yellow color is inversely proportional to the concentration of 25(OH)-vitamin D.

A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standard. 25(OH)-vitamin D in the samples is determined from this curve.

6.2 Test Procedure

1. **PRIOR TO USE IN THE ASSAY ALLOW ALL REAGENTS AND SAMPLES TO COME TO ROOM TEMPERATURE (18 - 26 °C). FOR THIS PURPOSE, OPEN THE KIT, TAKE OUT THE NEEDED INDIVIDUAL COMPONENTS AND MIX GENTLE, AVOIDING FOAM FORMATION**
2. MARK THE POSITIONS OF STD (STANDARDS)/SAMPLE/CTRL (CONTROL) ON A PROTOCOL SHEET
3. LABEL V-TUBES (E.G. 1.5 ML EPPENDORF-TUBES)
4. PIPETTE **30 µL OF STD** (STANDARD)/**SAMPLE/CTRL** (CONTROL) RESPECTIVELY, INTO THE CORRESPONDING TUBE
5. **RECONSTITUTE RELREAG** (RELEASING REAGENT) (SEE CHAPTER 5)
6. ADD **300 µL OF RELREAG** (RELEASING REAGENT) INTO EACH TUBE, VORTEX SHORTLY
7. INCUBATE FOR 1 HOUR AT 37 °C IN A WATER BATH OR HEATING BLOCK (DO NOT USE AN INCUBATOR) BEFORE STARTING THE INCUBATION, ENSURE THAT THE TEMPERATURE OF THE WATER BATH OR HEATING BLOCK HAS REACHED 37 °C AND IS MAINTAINED CONSTANT DURING THE INCUBATION
8. OPEN TUBES CAREFULLY AND ADD 600 µL **SAMDIL** (SAMPLE DILUTION BUFFER). CLOSE THE TUBES AND VORTEX CAREFULLY.
9. TAKE MICROTITER STRIPS OUT OF THE MICROTITER MODULE. UNUSED STRIPS MUST BE **COVERED** WITH THE ENCLOSED FOIL, STORED AT 2-8° C AND USED WITHIN 4 WEEKS
10. TRANSFER **50 µL OF STD** (STANDARD)/**SAMPLE/CTRL** (CONTROL) FROM THE V-TUBES TO RESPECTIVE WELL
11. ADD **150 µL OF AB** (ANTI 25(OH)-VITAMIN D ANTIBODY) INTO EACH WELL
12. COVER THE PLATE TIGHTLY WITH THE ENCLOSED FOIL AND **INCUBATE OVER NIGHT** (MIN. 18 – MAX. 22 HOURS) **AT 8-10 °C** IN THE DARK
13. ASPIRATE AND WASH THE WELLS **5X WITH 250 µL** OF DILUTED WASH BUFFER. THE USE OF **8-CHANNEL PIPETTE** IS RECOMMENDED. REMOVE REMAINING WASH BUFFER BY HITTING THE PLATE AGAINST PAPER TOWEL AFTER THE LAST WASH.

    FOR TECAN AND DYNEX INSTRUMENTS A PROGRAMMING PROTOCOL CAN BE REQUESTED.
14. ADD **200 µL CONJ** (CONJUGATE) INTO EACH WELL
15. COVER THE PLATE TIGHTLY WITH THE ENCLOSED FOIL AND **INCUBATE FOR 1 HOUR** AT

ROOM TEMPERATURE WHILE SHAKING

16. ASPIRATE AND WASH THE WELLS **5X WITH 250 µL** OF DILUTED WASH BUFFER. THE USE OF 8-CHANNEL PIPETTE IS RECOMMENDED. REMOVE REMAINING WASH BUFFER BY HITTING THE PLATE AGAINST PAPER TOWEL AFTER THE LAST WASH.
17. ADD **200 µL OF SUB** (SUBSTRATE) INTO EACH WELL
18. **INCUBATE FOR 10 - 15 MINUTES** AT ROOM TEMPERATURE (18-26°C) IN THE DARK
19. ADD **50 µL OF STOP** (STOP SOLUTION) INTO EACH WELL
20. DETERMINE THE **ABSORPTION** WITH AN ELISA READER **AT 450 NM.** IF THE HIGHEST EXTINCTION OF THE STANDARDS (STD) IS ABOVE THE RANGE OF THE PHOTOMETER, ABSORPTION MUST BE MEASURED IMMEDIATELY AT 405 NM AND THE OBTAINED RESULTS USED FOR EVALUATION. IF POSSIBLE, THE EXTINCTIONS FROM EACH MEASUREMENT SHOULD BE COMPARED WITH EXTINCTIONS OBTAINED AT A REFERENCE WAVELENGTH, E. G. 595 NM, 620 NM, 630 NM, 650 NM AND 690 NM CAN BE USED.

7 RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend the use of the "4-Parameter-algorithm".

1. 4-parameter-algorithm
   It is recommended a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e.g. 0.01).
2. Point-to-point-calculation
   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

8 LIMITATIONS

Samples with a 25-OH Vitamin D level greater than the highest calibrator should be diluted maximally 1+1 with ready-prepared 1x wash buffer (e. g. 50 µl sample + 50 µl 1x wash buffer) and re-assayed.

Whole blood is not suitable.

9 QUALITY CONTROL

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

9.1 PRECAUTIONS

- The quality control guidelines should be observed.
- Human material used in the kit components was tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Reagents of the kit package contain sodium azide and thimerosal as bactericides. Sodium azide and thimerosal are toxic. The substrates for the enzymatic color reactions are described to be also toxic and carcinogenic. Contact with skin or mucous membranes has to be avoided.
- Stop solution consists of sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water.

10 TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. DRG can therefore not be held responsible for any damage resulting from wrong use.

11 GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. DRG can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days of receipt of the product. The product shall be send to DRG together with the complaint in writing.

12 REFERENCES / LITERATURE

1. Scharla SH et al. (1996) Exp Clin Endocrinol Diabetes 104:289-292
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5. Scharla S (1997) Schattauer Verlag, Stuttgart, Seiten 217-242
6. Offermann G (1978) Dtsch med Wschr 103:1387-1388
7. Wielders JP, Wijnberg FA. (2009) Preanalytical stability of 25(OH)-vitamin D_3 in human blood or serum at room temperature: solid as a rock. Clin Chem. Aug;55(8):1584-5.

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