DRG® 25 (OH) Vitamin D (EIA-3153)

CE

RUO in the USA

Revised 1 Feb. 2011 rm (Vers. 8.1)

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.

INTENDED USE

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

MATERIAL SUPPLIED

1. Content	2.	3. Kit Components	4. Quantity
5. Microtiter Wells	**6. PLATE**	7. One holder with precoated strips	8. 12 x 8 wells
9. Wash Buffer	**10. WASBUF**	11. ELISA wash concentrate 10x	12. 1 x 100 mL
13. Assay Buffer	**14. ASYBUF**	15. Assay Buffer, ready to use	16. 1 x 15 mL
17. Binding Protein	**18. VDBP**	19. Vitamin D binding protein (VDBP), lyophilized (blue cap)	20. 1 vial
21. Precipitation Reagent	**22. PREC**	23. Precipitation Reagent (blue solution) ready to use	24. 1 x 50 mL
25. Antibody	**26. AB**	27. Anti-VDBP antibody, (yellow solution), ready to use	28. 1 x 11 mL
29. Standard, NSB	**30. STD**	31. Standards and NSB, ready to use (white cap) 32. (0; 6.4; 16; 40; 100; 250 nmol/L)	33. 7 vials
34. Control A & B	**35. CTRL**	36. Controls, ready to use (see specification for range)	37. 2 vials
38. Conjugate	**39. CONJ**	40. Peroxidase labeled conjugate, ready to use	41. 1 x 22 mL
42. TMB Substrate Solution	**43. SUB**	44. TMB substrate (Tetramethylbenzidine)	45. 2 x 15 mL
46. Stop Solution	**47. STOP**	48. ELISA stop solution, ready to use	49. 1 x 15 mL

CE

RUO in the USA

Revised 1 Feb. 2011 rm (Vers. 8.1)

MATERIAL REQUIRED BUT NOT supplied

- Bidistilled water (aqua bidest.)
- Laboratory balance
- Deep freezer -20 °C
- Precision pipettors calibrated to deliver 10-1000 µL
- Horizontal microtiter plate shaker
- Multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc. (one time products)
- Microtiter plate reader 450 nm (reference wave length 620 or 690 nm)

PREPARATION AND STORAGE OF REAGENTS

- To run the assay more than one time, make sure that the reagents are carefully stored at conditions stated on the label. Prepare just the appropriate amount necessary for the assay. The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with the less than **100 µL volume** should be centrifuged before use to avoid loss of volume.
- The **WASHBUF** (wash buffer concentrate) should be diluted with aqua bidest. **1:10** before use (add 900 mL of a. bidest. to 100 mL concentrate), mix well.
  Crystals could occur due to high salt concentration in the stock solutions. The crystals have to be redissolved at room temperature or at 37°C using a water bath **before dilution of the buffer solutions**.
  The buffer concentrates are stable at 2-8°C until the expiry date stated on the label.
  Diluted **buffer solution** could be stored in a closed container at **2-8°C for 1 month**.
- Incubate the **ASYBUF** (Assay Buffer) for **10 minutes at 37 °C** in a water bath before use.
- Dissolve **vitamin D binding protein** (VDBP, blue cap) in **11 mL** of Assay Buffer, mix well (no vortexer) and leave at room temperature for 30 min.
  Reconstituted VDBP is stable at -20°C until expiry date stated on the label. Avoid freezing/thawing cycles. We recommend to aliquote the VDBP (use glass containers). Frozen VDBP have to incubate for 10 minutes at 37 °C in a water bath before use. Avoid repeated freeze-thaw cycles!
- 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**.

SPECIMEN COLLECTION AND PREPARATION

1. Vitamin D is an inert substance. The samples can be stored at room temperature but we recommend storage at 2-8°C after collection when the measurement will be performed within the first 24 hours. Otherwise, the samples have to be stored at -20°C. Avoid repeated freeze-thaw cycles.

2. **Hemolysis** does not disturb the results; whole blood is not suitable.
3. Untreated **lipemic** samples lead to wrong results. Therefore we recommend:
   a. Centrifuge lipemic samples 10 min at 3.000 x g.
      The lipid phase floats up. Collect the aqueous phase.
   b. For highly lipemic samples we recommend using a special delipidation kit (REF.: K1003A). This kit was developed for lipemic samples in clinical laboratories.
1. Indicated incubation times and temperatures have to be observed exactly.
2. Mix the samples well before use.

ASSAY PROCEDURE

Principle of the test

This test kit is a competitive protein binding assay for the measurement of 25-OH Vit D. It is based on the competition of 25-OH Vit D present in the sample with 25-OH Vit D tracer, for the binding pocket of vitamin D binding protein (VDBP, Gc-globulin). Since all circulating 25-OH Vit D is bound to VDBP in vivo, samples have to be precipitated with precipitation reagent to extract the analyte. The supernatant can be used without further treatment within the test.
In the first incubation step, sample, calibrator, control, the vitamin D binding protein and the VDBP-Antibody, an antibody specific for this protein is added to the solid phase.
25-OH Vit D present in the sample then competes with the tracer, coated on the well for the specific binding site of the binding protein and the VDBP Antibody is bound to the vitamin binding protein. Hence, with increasing concentrations of 25-OH Vit D in the sample, the amount of binding protein, immobilized to the well via the tracer, is reduced. After a washing step to remove unbound components, the quantitation of VDBP is achieved by incubation with a host specific peroxidase labeled antibody using TMB (tetramethylbenzidine) as enzyme substrate. An acidic stopping solution is then added to stop the reaction. The colour converts to yellow. The intensity of the yellow colour is indirectly proportional to the concentration of 25-OH Vitamin D in the sample. A dose response curve of the absorbance unit vs. concentration is generated using the results obtained from the calibrators. Concentrations of 25-OH Vitamin D, present in the samples, are determined directly from this curve.

Extraction Procedure

Sample, Calibrator, NSB and Control preparation:

- Add **50 µL**, standard, control, NSB (non specific binding control, assay buffer only) and samples into plastic V tubes (we recommend Eppendorf tubes with a cap)
- Add **400 µL** of precipitation reagent to standard, control, NSB and samples. Mix thoroughly on a Vortex mixer. Incubate for 30 min at -20 °C.
- Centrifuge for 10 min at 3000 x g and at 4 °C. The supernatant contains the extracted 25-OH Vit D and must be assayed immediately.
  Please note: Keep samples during the transfer to the microtiterplate at 4 °C

CE

RUO in the USA

Revised 1 Feb. 2011 rm (Vers. 8.1)

We recommend reconstitution of VDBP at room temperature for 30 min. This can be done during the incubation of the samples.

Test Procedure

1. Prior to use in the assay allow all reagents and samples to come to room temperature (18 - 26 °C) and mix gentle, avoid foam formation.
2. Mark the positions of STD (Standards)/SAMPLE/CTRL (Control) on a protocol sheet.
3. Take microtiter strips out of the kit. Store unused strips covered at 2-8° C. Strips are stable until the expiry date stated on the label.
4. Put the **microtiter wells and the precipitated STD/SAMPLE/CTRL on a cool block or ice**. The microtiter plate and the precipitated STD/SAMPLE/CTRL must be cooled during the complete pipetting step.
5. Add **20 µL of STD/SAMPLE/CTRL** (Standard/Sample/Control) in duplicate into respective well.
6. Add **100 µL VDBP (binding protein)** into each well, exept the NSB.
7. Add **100 µL ASYBUF** (Assay Buffer) to the NSB wells.
8. Add **100 µL AB** (anti-VDBP antibody) into each well.
9. Cover the plate tightly and **incubate for 3 hours** at 8 - 10 °C in the dark.
10. Aspirate and wash the wells **5x with 250 µL** of diluted Wash Buffer**,** remove remaining Wash Buffer by hitting the plate against paper towel after the last wash.
11. Add **200 µL CONJ** (Conjugate) into each well.
12. Cover the plate tightly and **incubate for 1 hour** at 8 - 10 °C in the dark.
13. Aspirate and wash the wells **5x with 250 µL** of diluted Wash Buffer), remove remaining Wash Buffer by hitting the plate against paper towel after the last wash.
14. Add **200 µL of SUB** (Substrate) into each well.
15. **Incubate for 20 - 30 minutes** at room temperature (18-26°C) in the dark.
16. Add **50 µL of STOP** (Stop Solution) into each well, shake well.
17. Determine the absorption with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wave length is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm (690 nm) as a reference.

RESULTS

The following algorithms can be used alternatively to calculate the results.
However, we recommend to use the option 1: 4-parameter-algorithm.

CE

RUO in the USA

Revised 1 Feb. 2011 rm (Vers. 8.1)

1. *4-parameter-algorithm*
   It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard has to be specified with a value smaller than 1 (e. g. 0.01).
2. *Point-to-point-calculation*
   We recommend for the optical density a linear ordinate and for the concentration a linear abscissa.
3. *Spline-algorithm*
   We recommend for the optical density a linear ordinate and for the concentration a logarithmic abscissa. When using a logarithmic abscissa, the zero standard has to be specified with a value smaller than 1 (e.g. 0.01).
   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 manual control of the paired values should be made.

50. Standards	51. B/BO Mean [%]	52. B/BO Median [%]	53. SD B/BO [%] n = 17
54. NSB	55.	56.	57.
58. 1	59. 100	60. 100	61. 0
62. 2	63. 85	64. 86	65. 5.7
66. 3	67. 70	68. 69	69. 6.9
70. 4	71. 40	72. 42	73. 9.2
74. 5	75. 22	76. 22	77. 4.5
78. 6	79. 13	80. 14	81. 3.6

QUALITY CONTROL

Control samples or serum pools should be analyzed with each run of calibrators and samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the sample may not be valid.

Note

The vitamin D production in the skin is high variable and depends on the season- and daily time, degree of latitude, age, sun protection etc. The normal ranges depend on the method used (e. g. vitamin-D-release from the vitamin D binding protein, DBP) and serve only as orientation.

Literature references

Visser M, Deeg DJ, Puts MT, Seidell JC, Lips P. (2006) Low serum concentrations of 25-hydroxyvitamin D in older persons and the risk of nursing home admission. Am J Clin Nutr. Sep;84(3):616-22; quiz 671-2
Grant WB, Holick MF.Benefits and requirements of vitamin D for optimal health: a review. (2005) Altern Med Rev. Jun;10(2):94-111. Review
Wicherts IS, van Schoor NM, Boeke AJ, Visser M, Deeg DJ, Smit J, Knol DL, Lips P. (2007) Vitamin D status predicts physical performance and its decline in older persons. J Clin Endocrinol Metab. Jun;92(6):2058-65

CE

RUO in the USA

Revised 1 Feb. 2011 rm (Vers. 8.1)

PRECAUTIONS

- For research use only.
- 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.

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 the volumes of the different components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the test results. DRG can therefore not be held responsible for any damage resulting from this.

GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and volumes of the different 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 this.
- 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.

CE

RUO in the USA

Revised 1 Feb. 2011 rm (Vers. 8.1)

Literature

1. Scharla SH, et al. Exp Clin Endocrinol Diabetes 1996; 104:289-292
2. Scharla SH, et al. Osteoporos Int 1998;8 (Supplement 2) : S7-S12
3. Chapuy MC, et al. J Clin Endocrinol Metab 1996;81 : 11 29-33
4. Oster P et al. Akt Gerontol 1983; 13:221-2
5. Scharla S. Schattauer Verlag, Stuttgart, 1997; Seiten 217-242
6. Offermann G. Dtsch med Wschr. 1978; 103:1387-1388