25-OH-Vitamin D direct ELISA

Enzyme immunoassay for the quantitative direct determination of 25-OH-Vitamin D in human serum and plasma.

REF UK51081

Σ 12x8

2-8°C

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Intended Use
For In Vitro Diagnostic Use

The IDS 25-Hydroxy Vitamin D EIA kit is an enzymeimmunoassay intended for the quantitative determination of 25-hydroxyvitamin D (25-OH D) and other hydroxylated metabolites in human serum or plasma. Results are to be used in conjunction with other clinical and laboratory data to assist the clinician in the assessment of vitamin D sufficiency.

Summary and Explanation
Vitamin D is a commonly used collective term for a family of closely related seco-steroids. Upon exposure to sunlight, 7-dehydro-cholesterol, located deep in the actively growing layers of the epidermis, undergoes photolytic cleavage of the “B” ring to yield pre-vitamin D₃ which is isomerised to vitamin D₃ (cholecalciferol). Vitamin D₃ and vitamin D₂ (ergocalciferol) may also be obtained by dietary supplementation or from a limited number of foods. Vitamin D₂ is metabolised in a similar way to vitamin D₃.

Vitamin D is stored in adipose tissue and enters the circulation bound to vitamin D binding protein (VDBP) and albumin. In the liver, vitamin D is hydroxylated to give 25-hydroxyvitamin D which also circulates as a complex with VDBP. A small proportion of the 25-OH D is further hydroxylated in the kidney, under direct regulation by parathyroid hormone and ionised calcium levels, to form the biologically-active calcitropic hormone 1,25-di-hydroxyvitamin D. Further hydroxylation and metabolism of vitamin D produces compounds that are water soluble and readily excreted.

Hepatic vitamin D 25-hydroxylase activity is not tightly regulated, and changes in cutaneous production of vitamin D₃, or ingestion of vitamin D (D₃ or D₂), will result in changes in circulating levels of 25-OH D[1].

Serum concentration of 25-OH D is considered to be the most reliable measure of overall vitamin D status and thus can be used to determine whether a patient is vitamin D sufficient[2]. Assessment of vitamin D status may be required to determine the cause of abnormal serum calcium concentrations in patients.

Method Description
The IDS 25-Hydroxy Vitamin D EIA kit is an enzymeimmunoassay for the quantitation of 25-OH D and other hydroxylated metabolites in serum or plasma. Calibrators, controls and samples are diluted with biotin labelled 25-OH D. The diluted samples are incubated in microtitre wells which are coated with a highly specific sheep 25-OH D antibody for 2 hours at room temperature before aspiration and washing. Enzyme (horseradish peroxidase) labelled avidin, is added and binds selectively to complexed biotin and, following a further wash step, colour is developed using a chromogenic substrate (TMB). The absorbance of the stopped reaction mixtures are read in a microtitre plate reader, colour intensity developed being inversely proportional to the concentration of 25-OH D.

Warnings and Precautions
The IDS 25-Hydroxy Vitamin D EIA kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IDS Limited will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of non-compliance with the instructions provided.

CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Human serum: Calibrators [CAL] and Controls [CTRL]
Human material used in the preparation of this product has been tested by FDA recommended assays for the presence of antibody to Human Immunodeficiency Virus (HIV I and II), Hepatitis B surface antigen, antibody to Hepatitis C, and found negative. As no test can offer complete assurance that infectious agents are absent, the reagents should be handled in accordance at Biosafety Level 2.

Sodium azide
Xn. Harmful: Calibrators [CAL] and Controls [CTRL] contain sodium azide (NaN₃) >0.1% (w/w) (<1%).
R22 Harmful if swallowed.
R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S46 If swallowed, seek medical advice immediately and show this container or label.
S36/37 Wear suitable protective clothing and gloves.
This material and/or its container must be disposed of as hazardous waste. Some reagents in this kit contain sodium azide as a preservative, which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

**0.5M hydrochloric acid**

Stop Solution [HCL] contains 0.5M hydrochloric acid. 
R36/38 Irritating to eyes and skin. 
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. 
S36/37 Wear suitable protective clothing and gloves.

**Tetramethylbenzidine**

TMB Substrate [SUBS] contains 3,3',5,5'-tetramethylbenzidine. 
R21/22 Harmful by contact with skin and if swallowed. 
S36/37 Wear suitable protective clothing and gloves.

**Preparation of Reagents**

**Calibrators [CAL] and Controls [CTRL]**: Calibrators [CAL] and Controls [CTRL] are supplied lyophilised. Reconstitute with 1 mL of distilled or deionised water, replace stopper and stand for 10-15 minutes at room temperature. Invert several times to ensure complete reconstitution. Store at 2-8°C.

**25-D Biotin Solution [25-D BIOTIN SOLN]**: 25-D Biotin Concentrate [25-D BIOTIN 50x] is supplied lyophilised. Add 10 mL of Buffer [BUF] to the bottle of lyophilised 25-D Biotin Concentrate [25-D BIOTIN 50x] (blue colour). Replace the stopper and stand for 10-15 minutes at room temperature. Invert several times to ensure complete reconstitution. Add the reconstituted 25-D Biotin Concentrate [25-D BIOTIN 50x] (10 mL) back into the bottle containing the remaining Buffer [BUF] Mix well by inversion. The 25-D Biotin Solution (50 mL) is green in colour. Mark the bottle “25-D Biotin Solution”. Store at 2-8°C.

**Wash Solution [WASHBUF SOLN]**: Add the contents of each bottle of Wash Concentrate [WASHBUF 20x] to 950 mL of distilled or de-ionised water and mix. Store at room temperature.
Procedure

Materials Provided

1. CAL 0 - 6 – Calibrators
   (REF AC-5701A - AC-5701G):
   Lyophilised buffered human serum containing 25-hydroxyvitamin D and <1% sodium azide (0.09% reconstituted). The exact value of each Calibrator is printed on the bottle label, 1 mL per bottle, 7 bottles per kit.

2. MICROPLAT - Antibody Coated Plate
   (REF AC-5702W):
   Microplate with 25-hydroxyvitamin D sheep polyclonal antibody linked to the inner surface of the polystyrene wells, 12 x 8 well strips in a foil pouch with desiccant.

3. 25-D BIOTIN 50x - 25-D Biotin Concentrate
   (REF AC-5703):
   Lyophilised buffer containing 25-hydroxyvitamin D labelled with biotin, and proprietary stabilisers, 1 mL per bottle.

4. BUF - Buffer
   (REF AC-5703B):
   Proprietary reagent for dissociating 25-hydroxyvitamin D from binding proteins, 50 mL per bottle.

5. ENZYMCONJ - Enzyme Conjugate
   (REF AC-5704):
   Phosphate buffered saline containing avidin linked to horseradish peroxidase, protein, enzyme stabilisers and preservative. 22 mL per bottle.

6. CTRL 1 - 2 – Controls
   (REF AC-5705A - AC-5705B):
   Lyophilised human serum containing 25-hydroxyvitamin D and <1% sodium azide (0.09% reconstituted), 1 mL per bottle, 2 bottles per kit.

7. SUBS - TMB Substrate
   (REF AC-SUBS):
   A proprietary aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide, 28 mL per bottle.

8. HCL - Stop Solution
   (REF AC-STOP):
   0.5M Hydrochloric Acid, 13 mL per bottle.

9. WASHBUF 20x - Wash Concentrate
   (REF AC-WASHL):
   Phosphate buffered saline containing Tween, 50 mL per bottle.

10. Adhesive Plate Sealer
    8 per kit.

11. Documentation
    Package Insert and QC report.

Materials Required but not Provided

1. Disposable 12 x 75 mm borosilicate glass or polypropylene tubes. Note: polystyrene tubes are not suitable. Do not reuse tubes.

2. Precision pipetting devices to deliver 25 µL and 200 µL.

3. Repeating pipettes to deliver 1 mL, e.g. Eppendorf Multipipette 4780, or similar.

4. Precision multi-channel pipettes to deliver 100 µL and 200 µL.

5. Vortex mixer.

6. Automatic microplate washer (optional).

7. Photometric microplate reader and data analysis equipment.
Assay Procedure
Reconstitute or prepare reagents as described in “Preparation of Reagents”.
1. Prepare labelled borosilicate glass or polypropylene tubes, one for each Calibrator [CAL], Control [CTRL] and sample [SPE]
2. Add 25 µL of each Calibrator [CAL], Control [CTRL] or sample to the appropriately labelled tubes.
3. Add 1 mL of 25-D Biotin Solution [25-D BIOTIN SOLN] to all tubes. Vortex thoroughly for 10 seconds.
4. Add 200 µL of each diluted Calibrator, Control or sample to the appropriate wells of the Antibody Coated Plate [MICROPLAT] in duplicate. Cover the plate with an adhesive plate sealer. Incubate at 18-25°C for 2 hours.
5. Wash all wells three times with Wash Solution [WASHBUF SOLN].
   a) Automatic plate wash: Set plate washer to dispense at least 300 µL of Wash Solution [WASHBUF SOLN] per well. Fill and aspirate for 3 cycles.
   b) Manual wash: Decant the contents of the wells by inverting sharply. Dispense 250 µL of Wash Solution [WASHBUF SOLN] to all wells. Decant and repeat twice. Tap the inverted plate firmly on absorbent tissue to remove excess Wash Solution [WASHBUF SOLN] before proceeding to the next step.
7. Repeat wash step 5.
   Note: TMB Substrate is easily contaminated. Only remove the required amount for the assay from the bottle. Dispose of unused TMB Substrate. Do not return to bottle.
9. Add 100 µL of Stop Solution [HCL] to all wells using a multichannel pipette.
10. Measure the absorbance of each well at 450 nm (reference 650 nm) using a microplate reader within 30 minutes of adding the Stop Solution.

Calibration
25-OH D Calibrators are standardised using U.V. quantification.

Quality Control
The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. Two kit controls are provided. The controls should be tested as unknowns. Quality Control charts should be maintained to follow the assay performance.

Calculation of Results
Calculate the percent binding (B/Bo%) of each calibrator, control and unknown sample as follows:

\[
B/Bo\% = \left(\frac{\text{mean absorbance}}{\text{mean absorbance for '0' calibrator}}\right) \times 100
\]

Prepare a calibration curve on semi-log graph paper by plotting B/Bo% on the ordinate against concentration of 25-hydroxyvitamin D on the abscissa. Calculate B/Bo% for each unknown sample and read values off the curve in nmol/L (nM).

Alternative data reduction techniques may be employed but users should confirm that the selected curve fit is appropriate and gives acceptable results. Smoothed spline or 4PL curve fits are recommended.

Conversion of Units:
\[
x \text{nmol/L} \quad \Rightarrow \quad y \text{ng/mL}
\]

\[
x \times 0.40 \Rightarrow \quad y = x \times 2.5
\]
### Sample Assay Data

This data is for illustration only and must not be used for the calculation of any sample result.

<table>
<thead>
<tr>
<th>Well</th>
<th>Description</th>
<th>Abs.</th>
<th>Mean Abs.</th>
<th>B/Bo%</th>
<th>Result (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>Calibrator 0</td>
<td>2.476</td>
<td>2.530</td>
<td>2.503</td>
<td>0 nmol/L</td>
</tr>
<tr>
<td></td>
<td>Calibrator 1</td>
<td>2.313</td>
<td>2.288</td>
<td>2.301</td>
<td>6.8 nmol/L</td>
</tr>
<tr>
<td></td>
<td>Calibrator 2</td>
<td>1.912</td>
<td>1.908</td>
<td>1.910</td>
<td>14 nmol/L</td>
</tr>
<tr>
<td></td>
<td>Calibrator 3</td>
<td>1.495</td>
<td>1.499</td>
<td>1.497</td>
<td>27 nmol/L</td>
</tr>
<tr>
<td></td>
<td>Calibrator 4</td>
<td>0.919</td>
<td>0.905</td>
<td>0.912</td>
<td>67 nmol/L</td>
</tr>
<tr>
<td></td>
<td>Calibrator 5</td>
<td>0.521</td>
<td>0.522</td>
<td>0.522</td>
<td>179 nmol/L</td>
</tr>
<tr>
<td></td>
<td>Calibrator 6</td>
<td>0.372</td>
<td>0.368</td>
<td>0.370</td>
<td>380 nmol/L</td>
</tr>
<tr>
<td>H1, H2</td>
<td>Sample 1</td>
<td>1.237</td>
<td>1.257</td>
<td>1.247</td>
<td>49.8</td>
</tr>
<tr>
<td>A3, A4</td>
<td>Sample 2</td>
<td>0.951</td>
<td>0.969</td>
<td>0.960</td>
<td>38.4</td>
</tr>
<tr>
<td>B3, B4</td>
<td>Sample 3</td>
<td>0.591</td>
<td>0.612</td>
<td>0.602</td>
<td>24.0</td>
</tr>
</tbody>
</table>

### Typical Calibration Curve

This sample calibration curve is for illustration only.
Limitations of Use
Samples suspected of containing analyte concentrations in excess of the highest calibrator should be assayed in dilution.

As in the case of any diagnostic procedure results must be interpreted in conjunction with the patient’s clinical presentation and other information available to the physician.

The following substances have been tested and found not to interfere in the IDS 25-Hydroxy Vitamin D assay:

- Haemoglobin tested up to 1470 mg/dL
- Bilirubin tested up to 513 μmol/L
- Lipid tested up to 5.6 mmol/L triglyceride

Expected Values
The following range has been determined using the IDS 25-Hydroxy Vitamin D EIA kit and is provided for guidance only. Each laboratory should determine ranges for their local population.

Normal adults 47.7 - 144 nmol/L (n = 36)

Performance Data

Accuracy
The IDS 25-Hydroxy Vitamin D EIA kit was compared against a recognised radioimmunoassay for the quantitative determination of 25-hydroxyvitamin D and other hydroxylated metabolites. A population of 180 samples, selected to represent a wide range of 25-hydroxyvitamin D [9.3 - 151.2 nmol/L], were assayed by each method. Least squares regression analysis was performed on the comparative data: 

\[ \text{IDS} = 1.01(x) + 0.7; \text{ correlation coefficient}\ (r) = 0.91 \]

Sensitivity
The sensitivity, defined as the concentration corresponding to the mean minus 2 standard deviations of 10 replicates of the zero calibrator, is 5 nmol/L.

Precision

<table>
<thead>
<tr>
<th></th>
<th>Intra assay (nmol/L)</th>
<th>Inter assay (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>% CV</td>
</tr>
<tr>
<td>A</td>
<td>122</td>
<td>97</td>
</tr>
<tr>
<td>A</td>
<td>95.6</td>
<td>97</td>
</tr>
<tr>
<td>B</td>
<td>147</td>
<td>104</td>
</tr>
<tr>
<td>B</td>
<td>123</td>
<td>105</td>
</tr>
<tr>
<td>Mean</td>
<td>101</td>
<td></td>
</tr>
</tbody>
</table>

Recovery
Recovery was assessed by adding 25-OH D to samples prior to assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measured (nmol/L)</th>
<th>Expected (nmol/L)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>122</td>
<td>126</td>
<td>97</td>
</tr>
<tr>
<td>A</td>
<td>95.6</td>
<td>98.4</td>
<td>97</td>
</tr>
<tr>
<td>B</td>
<td>147</td>
<td>141</td>
<td>104</td>
</tr>
<tr>
<td>B</td>
<td>123</td>
<td>118</td>
<td>105</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>101</td>
</tr>
</tbody>
</table>

Linearity
Linearity was assessed by diluting samples with buffer (PBS containing 9%BSA) prior to assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measured (nmol/L)</th>
<th>Expected (nmol/L)</th>
<th>% M/Exp</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>83.9</td>
<td>08.1</td>
<td>100</td>
</tr>
<tr>
<td>A/2</td>
<td>41.0</td>
<td>21.0</td>
<td>99</td>
</tr>
<tr>
<td>A/4</td>
<td>20.8</td>
<td>10.5</td>
<td>125</td>
</tr>
<tr>
<td>A/8</td>
<td>13.1</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>83.9</td>
<td>42.0</td>
<td>104</td>
</tr>
<tr>
<td>B/4</td>
<td>23.1</td>
<td>21.0</td>
<td>110</td>
</tr>
<tr>
<td>B/8</td>
<td>10.7</td>
<td>10.5</td>
<td>102</td>
</tr>
<tr>
<td>C</td>
<td>104</td>
<td>52.0</td>
<td>88</td>
</tr>
<tr>
<td>C/2</td>
<td>45.9</td>
<td>26.0</td>
<td>87</td>
</tr>
<tr>
<td>C/4</td>
<td>22.5</td>
<td>13.0</td>
<td>108</td>
</tr>
<tr>
<td>C/8</td>
<td>14.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>102</td>
</tr>
</tbody>
</table>

Specificity
The specificity of the antiserum was assessed with the following analytes at 50% binding of the zero calibrator.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-Hydroxyvitamin D₃</td>
<td>100%</td>
</tr>
<tr>
<td>25-Hydroxyvitamin D₂</td>
<td>75%</td>
</tr>
<tr>
<td>24,25-Dihydroxyvitamin D₃</td>
<td>≥100%</td>
</tr>
<tr>
<td>Cholecalciferol (D₃)</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Ergocalciferol (D₂)</td>
<td>&lt;0.30%</td>
</tr>
<tr>
<td>Symbols</td>
<td>Symbole</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>REF</td>
<td>Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:</td>
</tr>
<tr>
<td>LOT</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός-Παραγωγή:</td>
</tr>
<tr>
<td>Use by:</td>
<td>Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:</td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα</td>
</tr>
<tr>
<td>LYO</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Λυοφιλισµένο</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipamiento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.</td>
</tr>
<tr>
<td>Keep away from heat or direct sun light.</td>
<td>Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται µακριά από θερµότητα και άµεση επαφή µε το φως του ηλίου.</td>
</tr>
<tr>
<td>Keep at:</td>
<td>Lagern bei: / Stocker à: / Almacenar a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
</tr>
<tr>
<td>Manufacturer:</td>
<td>Hersteller: / Fabricant: / Productor: / Fabricante: / Fabricante: / Παραγωγός:</td>
</tr>
<tr>
<td>Caution!</td>
<td>Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!</td>
</tr>
</tbody>
</table>

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύµβολα των συστατικών του κιτ συµβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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| WEB: http://www.ibl-transatlantic.com |

**LIABILITY:** Complaints will only be accepted in written and if all details of the test performance and results are included (complaint form available from IBL or supplier). Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.4 / 2008-03-12