



## 25-Hydroxy Vitamin D Assay

### Configuration

Diazyme's 25-Hydroxy (25-OH) Vitamin D Assay is provided in the following configuration (80 tests):

| Instrument      | REF      | Kit size   |
|-----------------|----------|--|
| Roche Modular P | DZ688C-K | Diluent: 1 x 17 mL<br>Reagent R1: 1 x 8.5 mL<br>Reagent R2: 1 x 17 mL<br>Reagent R3: 1 x 8.5 mL<br>Calibrators: 5 x 1 mL |

### Intended Use

The Diazyme 25-OH Vitamin D Assay is designed for the quantification of total 25-OH Vitamin D in serum and plasma samples.

### Background

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, osteomalacia, cancers, and cardiovascular diseases. 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. Vitamin D deficient patients who are prescribed a daily Vitamin D supplement should regularly monitor their serum or plasma Vitamin D levels in order to reach an optimal level and prevent their 25-OH Vitamin D concentrations from reaching excessive levels that are considered toxic<sup>1-5</sup>.

### Test Principle

The test is based on the principle of  $\alpha$ -complementation of the enzyme  $\beta$ -galactosidase and the competition between an enzyme donor-25-OH Vitamin D conjugate, an anti-Vitamin D antibody and the 25-OH Vitamin D content of a serum sample. Samples with higher 25-OH Vitamin D concentrations produce higher  $\beta$ -galactosidase activities and *vice versa*. A nitrophenyl- $\beta$ -galactoside derivative (NPG) is used as the enzyme substrate. The reaction's product has maximum absorbance at 415 nm. The 25-OH Vitamin D concentration of a sample is proportional to the measured  $\beta$ -galactosidase activity.

### Reagent – "Working Solutions"

**DILUENT**: Dilution buffer with stabilizers.

**REAGENT 1**: Dissociation solution, substrate and stabilizers.

**REAGENT 2**: Enzyme Donor-Vitamin D conjugate and stabilizers.

**REAGENT 3**: Enzyme Acceptor and stabilizers.

**CALIBRATOR 1 – 5**: Human serum containing specific amounts of 25-OH Vitamin D and stabilizers.

### Precautions

1. USA: For Research Use Only. Not for in vitro diagnostic use.
2. EU: IVD
3. **DO NOT INGEST**. Avoid contact with skin and eyes.
4. **REAGENT** contain sodium azide, which may react with lead or copper plumbing to form explosive compounds. Flush drains with copious amounts of water when disposing of reagents.
5. Specimens containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories, 5<sup>th</sup> Ed (HHS Publication Number [CDC] 21-1112).
6. **CALIBRATOR** and **CONTROL** contain human source material. Each donor unit of serum in the preparation of these materials were tested by FDA-approved methods and found negative for the Human Immunodeficiency Virus Antibody (HIV I/II Ab), Hepatitis B Surface Antigen (HBsAg), and Hepatitis C Virus Antibody (HCV). Because no method can offer complete assurance as to the absence of infectious agents, this material and all samples should be handled as though capable of transmitting infectious disease and such biohazardous material should be disposed of according to relevant local, state or federal regulations.
7. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768 or email [support@diazyme.com](mailto:support@diazyme.com).

### Reagent Storage and Stability

#### Calibrators and Controls

**CALIBRATOR** and **CONTROL** are serum-based solutions and are stable when stored at 2-8°C until the expiration date on the label.

Mix vials well before assaying.

#### Diluent:

This solution is ready to use and is stable when stored at 2-8°C until the expiration date on the label. After use, the solution should be kept capped in its reagent bottle.

#### Reagents 1, 2 and 3

The **REAGENTS** is ready to use and are stable when stored at 2-8°C until the expiration date on the label. After use, the **REAGENTS** should be kept capped in their corresponding reagent bottles.

### Specimen Collection and Handling

Serum, heparinized plasma or EDTA plasma samples can be used for the assay. For serum, collect whole blood by venipuncture and allow clotting. For plasma, mix the sample by gentle inversion prior to centrifugation. Centrifuge and separate serum or plasma as soon as possible after collection. Do not use hemolysed samples.

The specimens may be refrigerated at 2-8°C for two weeks. For long term storage, they can be stored at -20°C. Avoid repeated freeze-thaw cycles. Samples showing clear signs of hemolysis should not be used because excess hemoglobin (> 100 mg/dL) may interfere with the assay's results.

Allow the refrigerated or frozen-thawed samples to equilibrate to room temperature for 30 minutes before use; samples must be mixed well before analysis.

### Materials Provided

Please see "Reagents – 'Working Solutions'" section.

## Materials Required but not Provided

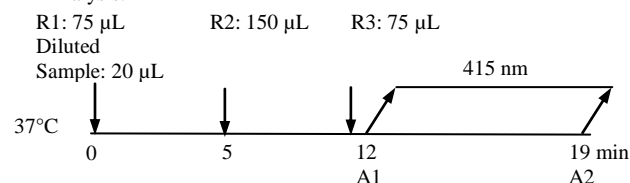
The Diazyme 25-OH Vitamin D Control Set (REF DZ688C-CON) contains human serum containing specific amounts of 25-OH Vitamin D. CONTROL material is supplied in aliquots of 1.0 mL. CONTROL 1 has a concentration of 25-OH Vitamin D that corresponds to an insufficient sample. CONTROL 2 has a concentration of Vitamin D that corresponds to a sufficient sample. The Control Set is sold as 2 x 1 mL vials.

## Test Procedure

The assay procedure for the Roche Modular P chemistry analyzer is shown below:

- Specimen (CALIBRATOR, CONTROLS and samples) are first diluted on-board: 20 µL of serum are diluted with 155 µL of Diluent. 20 µL of the diluted specimen is then used for analysis.

- Analysis:



Full application parameters sheet for Roche Modular P is included in the kit. The above scheme should be used as a guideline to develop assay parameters on similar chemistry analyzers.

Questions regarding additional applications of the assay can be answered by phone at 858-455-4768 or by email at [support@diazyme.com](mailto:support@diazyme.com).

## Calibration

The Diazyme 25-OH Vitamin D Calibrator Set (REF DZ688C-CAL) is a five calibrator set that is provided in the kit.

### Calibration Frequency

The CALIBRATOR should be used to calibrate the assay before each run.

## Quality control

We recommend that each laboratory use the Diazyme 25-OH Vitamin D Control Set to validate the performance of REAGENTS. These controls can be purchased separately.

## Results

Results are expressed in ng/mL. **Note:** Samples with values greater than 143 ng/mL should be reported as >143 ng/mL.

## Reference Range

There is little universal agreement on the optimal concentration of 25-OH Vitamin D. Review of literature<sup>1-6</sup> suggests the recommendation for 25-OH Vitamin D levels are:

| Level              | Range (ng/mL) |
|--------------------|---------------|
| Deficient          | <10           |
| Insufficient       | 10-29         |
| Sufficient         | 30-100        |
| Potential Toxicity | >100          |

## Limitations

- The assay is designed for use with serum and plasma samples only.
- Samples suspected of containing analyte values greater than 143 ng/mL should be reported as >143 ng/mL.
- As with any 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.

## Analytical Characteristics

Results obtained from individual laboratories may vary.

### Sensitivity

The limit of blank (LoB) of the assay was 2.0 ng/mL. The limit of detection (LoD) of the assay was found to be 3.5 ng/mL. The limit of quantitation (LoQ) of the assay was found to be 7.6 ng/mL.

### Accuracy

The performance of this assay was compared to the performance of a legally marketed 25-OH Vitamin D enzyme immunoassay. Using 60 serum samples with 25-OH Vitamin D concentrations ranging from 8.1 ng/mL to 139.4 ng/mL, the R<sup>2</sup> correlation coefficient between the two methods was 0.963, the slope was 0.912 and the y intercept was +5.645 ng/mL.

### Precision

For insufficient and sufficient serum samples, within-run CVs were 4.3% to 8.7%. Between-run CVs were 6.0% to 9.1%. For deficient samples (<10 ng/mL), within-run CVs were 10% to 12.7%, and between-run CVs were 13.1 to 16.5%.

### Linearity

The assay was found to be linear between 7.6 and 143 ng/mL.

### Interference

The assay's results were not significantly affected by the presence of bilirubin (up to 40 mg/dL for free bilirubin and up to 40 mg/dL of conjugated bilirubin), hemoglobin (up to 100 mg/dL), ascorbic acid (up to 10 mM) and triglycerides (up to 750 mg/dL).

## References

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