Bioway Chemistry Reagent Series

25-Hydroxy Vitamin D Reagent Kit

Detection of 25-Hydroxy Vitamin D in Human Serum or plasma on Chemistry Analyzers

Cat.No. R073K11 25-OH Vitamin D Reagent Kit

SUMMARY OF TEST PROCEDURE

Table 1: Instrument Parameters*

<table>
<thead>
<tr>
<th>Calibration method</th>
<th>5 point non-linear</th>
<th>Sample volume</th>
<th>20 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>415 nm</td>
<td>R1 volume</td>
<td>75 µl</td>
</tr>
<tr>
<td>Test method</td>
<td>2 point end</td>
<td>R2 volume</td>
<td>150 µl</td>
</tr>
<tr>
<td>Reaction temperature</td>
<td>37°C</td>
<td>R3 volume</td>
<td>75 µl</td>
</tr>
</tbody>
</table>

*Refer to Figure 1 and the package insert for detail

INTENDED USE

Bioway Chemistry Reagent Series 25-Hydroxy Vitamin D Reagent Kit (the Kit) is an assay intended for in vitro quantitative detection of 25-OH Vitamin D in human serum or plasma on automated clinical chemistry analyzers.

SUMMARY AND EXPLANATION

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.

TEST PRINCIPLES

The assay is based on the principle of a complementation of the enzyme β-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 β-galactosidase activities and vice versa. A nitrophenyl-β-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 β-galactosidase activity.

MATERIALS PROVIDED

Reagents:

| R1 | Dissociation solution, substrate and stabilizers |

R2 | Enzyme Donor-Vitamin D conjugate and stabilizers
R3 | Enzyme Acceptor and Stabilizers

MATERIALS NEEDED BUT NOT PROVIDED

1. Automated chemistry analyzer.
2. 25-OH Vitamin D calibrator set (available for purchase) and control set (available for purchase).

INSTRUMENT

The Kit is applicable on most automated chemistry analyzers. Refer to specific instrument application for suggested settings.

STORAGE AND STABILITY

Store the reagents at 2-8°C. Avoid direct sunlight. The Kit is stable for 12 months when stored properly. R1, R2 and R3 reagents are stable for 1 month at 2-8°C after opening.

PRECAUTIONS

1. The Kit is for in vitro diagnostic use only. Not for use in humans or animals.
2. The instructions must be followed to obtain accurate results.
3. Do not use the reagents beyond the expiration date.
4. Do not mix and use different lots of reagents.
5. Treat all specimens as infectious. Proper handling and disposal procedures of specimens and test materials should be strictly followed.
6. Reagents contain sodium azide as preservative; avoid contact with skin and eyes, flush with copious amounts of water when disposing.

SPECIMEN COLLECTION AND HANDLING

Follow standard laboratory procedures to collect serum, EDTA or lithium heparin plasma samples. It is recommended to perform test immediately after sample collection to avoid hemolysis. If the test cannot be done immediately, store sample at 2-8°C for up to 2 weeks or at -20°C for up to 1 month. Avoid repeated freezing and thawing.

TEST PROCEDURE (see Figure 1)


Test procedure: see Figure 1 and Table 1 for instrument parameter setup. Refer to specific instrument application for suggested setting.
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Detection of 25-Hydroxy Vitamin D in Human Serum or plasma on Chemistry Analyzers

1. Add 20 µl sample and 75 µl R1; mix well and incubate at 37°C for 5 minutes.
2. Add 150 µl of R2, mix well and incubate at 37°C for 7 minutes.
3. Add 150 µl of R3 and Take continuous optical density measurement for 7 minutes.
4. Calculate average ΔOD

RESULT

The 25-OH Vitamin D value can be obtained by using the calculated ΔOD to find the corresponding value on a calibration curve prepared with known values.

EXPECTED VALUES

30-100 ng/mL.
It is recommended for each laboratory to establish its own expected values.

QUALITY CONTROL

Using Bioway 25-OH Vitamin D control is recommended before each batch of tests to ensure the test is properly performed and all reagents and the instrument are functional as specified.

LIMITATIONS

1. The Kit is for in vitro use on automated chemistry analyzers only.
2. Hemolysis samples may cause inconsistent results.
3. The test result from the Kit should not be used as the only basis for definite diagnosis.

PERFORMANCE CHARACTERISTICS

Linearity: 7.6-143 ng/mL (R²≥0.990)
Accuracy: Relative deviation ≤ 15%
Precision: Within Run: 4.3%≤CV≤8.7%;
Run-to-Run: 6.0%≤CV≤9.1%

Interference: no interference detected for: Triglyceride (750 mg/dL), Ascorbic Acid (10mM), Hemoglobin (100 mg/dL) and bilirubin (40 mg/dL).

Detection Limit: 7.6 ng/mL

REFERENCES


Not Intended for Sale in the United States.

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