DATA SHEET

DE1971 25-OH Vitamin D total ELISA

Immunoenzymetric assay for the in vitro quantitative measurement of 25-hydroxyvitamin D2 and D3 (25OH-D2 and 25OH-D3) in serum

Technology	: ELISA
Kit size	: 96 wells
Sample material	: serum
Sample preparation	: -
Sample volume	: 50 µl
Standard range	: 12 - 162 ng/ml
Incubation	: 2h, 30 min, 15 min (RT/shaking)
Measuring system	: TMB 450 nm
Sensitivity	: 1.5 ng/ml

Special remarks:

CLINICAL BACKGROUND

Vitamin D is the generic term used to designate Vitamin D2 or ergocalciferol and Vitamin D3 or cholecalciferol. Humans naturally produce Vitamin D3 when the skin is exposed to ultraviolet sun rays.

In the liver mainly, Vitamin D3 is metabolised into 25-Hydroxyvitamin D3 (25OH D3) which is the main form of Vitamin D circulating in the body. 25OH D3 is a precursor for other Vitamin D metabolites and has also a limited activity by itself. The most active derivative is 1,25-hydroxyvitamin D3, produced in the kidney (or placenta) by 1-hydroxylation of 25OH D3. 25OH Vitamin D stimulates the intestinal absorption of both calcium and phosphorus and also bone resorption and mineralisation.

25OH Vitamin D might also be active in other tissues responsible for calcium transport (placenta, kidney, mammary gland …) and endocrine gland (parathyroid glands, beta cells…). Vitamin D3 and Vitamin D2 are also available by ingestion through food or dietary supplementation.

As Vitamin D2 is metabolised in a similar way to Vitamin D3, both contribute to the overall Vitamin D status of an individual. It is the reason why it is very important to measure both forms of 25OH Vitamin D equally for a correct diagnosis of Vitamin D deficiency,

insufficiency or intoxication. Vitamin D deficiency is an important risk factor for rickets, osteomalacia, senile osteoporisis, cancer and pregnancy outcomes. The measurement of both 25OH Vitamin D forms is also required to determine the cause of abnormal serum calcium concentrations in patients. Vitamin D intoxication has been shown to cause kidney and tissue damages.

PRINCIPLES OF THE METHOD

The Demeditec 25OH Vitamin D Total ELISA is a solid phase Enzyme Linked Immunosorbent Assay performed on microtiterplates. During a first 2 hours incubation step, at room temperature, total 25OH Vitamin D (D_2 and D_3) present in calibrators, controls and samples is dissociated from binding serum proteins to fix on binding sites of a specific monoclonal antibody. After 1 washing step, a fixed amount of 25OH Vitamin D-labelled with biotin in presence of horseradish peroxidise (HRP), compete with unlabelled 25OH Vitamin D_2 and 25OH Vitamin D_3 present on the binding sites of the specific monoclonal antibody. After a 30 minutes incubation at room temperature, the microtiterplate is washed to stop the competition reaction. The Chromogenic solution (TMB) is added and incubated for 15 minutes. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is inversely proportional to the total 25OH Vitamin D (D_2 and D_3) concentration. A calibration curve is plotted and the total 25OH Vitamin D (D_2 and D_3) concentrations of the samples are determined by dose interpolation from the calibration curve.