25-OH Vitamin D ELISA

- Optimised and reliable detection of 25-OH vitamin D$_3$ and D$_2$
- Excellent correlation of the measurement results with reference methods (LC-MS/MS and HPLC)
- Fully automatable on all open ELISA platforms

Technical data

- **Coating**: Anti-25-OH vitamin D (monoclonal)
- **Calibration**: Quantitative, in nanograms per millilitre (ng/ml)
  - Calibrator 1: 0 ng/ml
  - Calibrator 2: 4 ng/ml
  - Calibrator 3: 10 ng/ml
  - Calibrator 4: 25 ng/ml
  - Calibrator 5: 60 ng/ml
  - Calibrator 6: 120 ng/ml
- **Sample dilution**: Serum or plasma; 20 µl; 1:26 in sample buffer containing biotin
- **Reagents**: Ready for use, with the exception of the wash buffer (10x) and biotin (100x). Colour-coded solutions
- **Test procedure**: 120 min / 30 min / 15 min (room temperature), fully automatable
- **Measurement**: 450 nm. Reference wavelength between 620 nm and 650 nm
- **Test kit format**: 96 break-off wells, kit includes all necessary reagents
- **Order number**: EQ.6411-9601

Clinical significance

There are two forms of vitamin D: vitamin D$_3$, which is mainly produced in the skin and vitamin D$_2$, which is acquired by consumption of vegetable foods. Both forms are bound to a binding protein in the blood stream and metabolised in the liver into 25-OH vitamin D$_2$ or D$_3$, a storage form. It is only in the kidney that the biologically active metabolite 1,25 dihydroxy vitamin D$_3$, which has the function of a hormone (D hormone) is synthesised. The D hormone regulates calcium intake from the intestine, bone mineralisation, differentiation of osteoblasts and synthesis of bone matrix.

Vitamin D deficiency is a worldwide problem with serious health consequences. The serum level of 25-OH vitamin D, which shows the highest concentration of all vitamin D metabolites due to its storage function, is the best indicator of the vitamin D supply of the human organism. Already a small vitamin D deficiency with a 25-OH vitamin D level of 12–30 ng/ml (30–75 nmol/l) in the blood leads to a reduced calcium intake and thus to a secondary increase in parathyroid hormone and increased bone resorption. A vitamin D deficiency is therefore one of the most important risk factors for senile osteoporosis. A severe vitamin D deficiency with a level of <12 ng/ml (<30 nmol/l) leads e.g. to the clinical image of rickets in children or osteomalacia in adults, which are characterised by disrupted bone formation or insufficient matrix mineralisation. Moreover, hypovitaminosis D constitutes a risk factor for numerous further diseases.

Diagnostic application

Alongside the reliable diagnosis of vitamin D deficiency, the 25-OH Vitamin D ELISA is also useful for monitoring of therapy, since the effect of drugs may vary and vitamin D deficiency syndromes require treatment over a longer period of time, often over years or even decades. As opposed to antibodies used in other commercial test systems, the novel monoclonal antibody used in this test is equally specific for both forms of 25-OH vitamin D (100%).
**Test principle**

25-OH vitamin D from the patient sample is bound by anti-25-OH antibodies on the microplate. Free antibody binding sites are occupied by labelled 25-OH vitamin D. The intensity of the colour formed after addition of the chromogen/substrate solution is measured using a photometer. The colour intensity is inversely proportional to the 25-OH vitamin D concentration in the serum or plasma.

**Reproducibility**

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 3 samples. The intra-assay CVs are based on 40 measurements for each serum and the inter-assay CVs on four measurements repeated in ten different runs.

**Reference range**

Sera from 359 healthy blood donors (aged 13–99 years) were investigated using the EUROIMMUN 25-OH Vitamin D ELISA. The mean 25-OH vitamin D concentration was 20.9 ng/ml (at 5th–95th percentile: 8.2–37.4 ng/ml). The vitamin D supply was optimal in 53 blood donors (30–70 ng/ml) and deficient in 306 blood donors (<30 ng/ml).

**Correlation of the EUROIMMUN 25-OH Vitamin D ELISA with HPLC, LC-MS/MS and IDS ELISA**

The EUROIMMUN 25-OH Vitamin D ELISA was compared with different reference tests and showed the following correlations: HPLC, \( r^2 = 0.91, n = 80 \); LC-MS/MS, \( r^2 = 0.93, n = 100 \); IDS 25-OH Vitamin D Direct EIA, \( r^2 = 0.93, n = 231 \). The correlation of the obtained results was very high.