Human IGFBP3 ELISA Kit

Enzyme Immunoassay for the quantification of human Insulin-like Growth Factor Binding protein-3 (IGFBP3) in serum and plasma

Catalog number: ARG80494

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Insulin-like growth factors (IGF)-I and-II are bound to specific binding proteins (IGFBPs) in the circulation. To date, at least six binding proteins can be distinguished on the basis of their amino acid sequence. They are designated as IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5 and IGPBP-6. Lately the discovery of a new IGFBP-7 has been discussed.

The predominating IGFBP in blood is IGFBP-3, which largely determines the total IGF-I and IGF-II concentration. In contrast to the other binding proteins, IGFBP-3 has the unique property to associate with an acid-labile non-binding subunit (ALS) after binding of either IGF-I or IGF-II. Most of the IGFBP-3 in plasma is present as the high molecular weight ternary complex, however, small amounts of free IGFBP-3 are also found. The development of specific immunoassays for IGFBP-3, those also recognize the complete high molecular weight complex, provided new in-sights into its regulation. On the basis of these findings serum IGFBP-3 has proved to be an additional useful test in the repertoire of diagnostic tools for evaluation of growth disorders.

Several factors besides GH influence IGFBP-3 levels: age including sexual development, nutrition, hypothyroidism, diabetes mellitus, liver function and kidney function. IGFBP-3 levels are decreased by malnutrition, although less than IGF-I, in hypothyroidism, in diabetes mellitus and in hepatic failure, but are increased in chronic renal failure. Measurement over 24 hours revealed constant circadian levels. For clinical practice, the most important regulatory

factor is GH. Single IGFBP-3 measurements correlate significantly with the logarithm of the integrated spontaneous GH secretion. In patients with GH deficiency, IGFBP-3 levels are subnormal and increase gradually to within the normal range after several days of GH administration. The slow response to GH and constant circadian levels during chronic daily application of GH suggest that IGFBP-3 reflects the GH secretory state over days.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for IGFBP-3 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IGFBP-3 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for IGFBP-3 is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of IGFBP-3 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm.The concentration of IGFBP-3 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C
Standards A-E (0.4, 2, 6, 15, 30 ng/ml)	5 vials	4°C, Lyophilized
Control 1 (1700 - 2500 ng/ml)	1 vial	4°C, Lyophilized
Control 2 (3300 - 5000 ng/ml)	1 vial	4°C, Lyophilized
HRP-Conjugate Antibody	12 ml	4°C
Dilution Buffer	30 ml	4°C
Sample Buffer	120 ml	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- Controls: Control 1 and 2, lyophilized, contain human Serum and should be reconstituted in 250 µl Sample Buffer each. Dilute 1:505-fold (or in respective dilution rate of the sample) with Sample Buffer.
- Standards: Reconstituting with 1 ml Sample Buffer each.
- Samples: Samples have to be diluted 1:505-fold (In general) with Sample Buffer.
  - Pipette 1 ml Sample Buffer in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 10 µL Serum- or Plasma (dilution factor 101). Add 400 µL Sample Buffer in another PE- /PP-tube and 100 µL of the thoroughly mixed first dilution (dilution factor 5). After mixing use 50 µL of this 1:505 diluted solution within 1 hour per determination in the assay.
  - Sample stability after dilution of the sample: maximum 1 hour at 20-25°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl Dilution Buffer** into all wells
3. Add **50 µl Sample Buffer** in duplicates into wells.
4. Add **50 µl** of **standards**, **controls** and **samples** in duplicate into wells.
5. Incubate the plate for **1 hour** at **RT**.
6. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add **100 µl** of **HRP-Conjugate** Antibody into each well. Cover wells and incubate for **1 hour** at **RT**.
8. Aspirate each well and wash as step 6.
9. Add **100 µl** of **TMB** Reagent to each well. Incubate for 30 minutes at **RT** in dark.
10. Add **100 µl** of **Stop Solution** to each well.
11. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

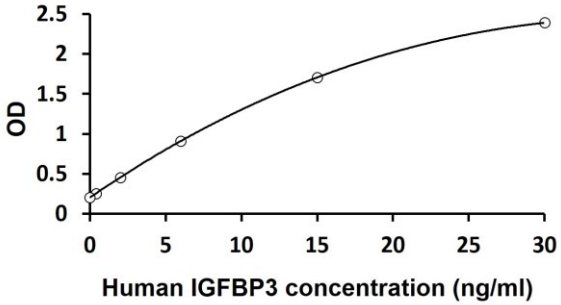
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. The IGFBP-3 concentration in ng/mL (or pg/mL, according the chosen unit for the standards) of the samples can be calculated by multiplication with the respective dilution factor (505).

EXAMPLE OF TYPICAL STANDARD CURVE



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of IGFBP-3 ranged from 0.4-30 ng/ml. The mean MDD was 0.1 ng/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.51% and inter-assay precision was 6.3%.