Catalog #80580

V.5

Crystal Chem
High Performance Assays

IGFBP-3 ELISA
Kit Instructions

For the quantitative determination of IGFBP-3 in
human serum and plasma

Catalog #80580
96 Assays

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

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A. Intended Use

The IGFBP-3 ELISA kit is for the quantitative determination of IGFBP-3 in human serum and plasma. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

IGFBP-3 (Insulin-like Growth Factor Binding Protein-3) is the most abundant IGFBP in circulation and therefore of special relevance in regulation of IGF effects. This is reflected by the indicative value of serum IGFBP-3 concentration in diagnostics of growth disturbances. IGFBP-3 has been shown to be able to induce apoptosis, promote tumor growth and inhibit cellular migration and metastasis dependant on tissue and tumor stage.

C. Principle of the Assay

The IGFBP-3 ELISA kit is an ELISA sandwich assay for IGFBP-3. It utilizes two specific and high affinity antibodies for this protein. IGFBP-3 in the sample binds to the first antibody coated on the microtiter plate. In the following step, the biotinylated and Streptavidin-Peroxidase conjugated second specific anti-IGFBP-3 antibody binds in turn to the immobilized IGFBP-3. In the closing substrate reaction, the IGFBP-3 levels of the samples can be measured by color intensity.

D. Kit Storage

1. Upon receipt of the IGFBP-3 ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
STD1-5	Standards	1 x 5 vials
CON1-2	Controls	1 x 2 vials
AB CONJ	Antibody HRP Conjugate	1 x 12 mL
SAM BUF	Sample Buffer	1 x 120 mL
DIL BUF	Dilution Buffer	1 x 30 mL
WASH	Wash Buffer (20X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL
	Sealing tape for plate	2x, adhesive

E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled or deionized water
Polypropylene microtubes
Volumetric flasks
Vortex mixer
Microplate shaker (350 rpm)
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the small sample volumes required (5 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each standard and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Serum and plasma samples collected with EDTA or Heparin anticoagulant can be used. Hemolytic samples should be avoided. Samples should be chilled as soon as possible after sample withdrawal. For long-term storage, samples can be stored for more than 2 years at -20°C. Avoid repeated freeze-thaw cycles of samples.

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture.
2. Standards 1-5
   Standards are provided in lyophilized form with concentrations ranging from 0.4 ng/mL to 30 ng/mL. Dilute each standard with 1 mL of Sample Buffer (marked "SAM BUF"). After reconstitution, it is recommended that standards be allowed to sit for 15 mins at room temperature and then mixed thoroughly but gently with a Vortex mixer. Reconstituted standards are stable for four weeks at -20°C. Standards should be not be repeatedly thawed, so standards should be appropriately aliquoted in appropriate volumes prior to being frozen. Standards are provided in the following concentrations: 0.4, 2, 6, 15, and 30 ng/mL.

3. Controls 1-2
   Controls are provided in lyophilized form with target value and ranges included on their labels. Dilute controls with 250 µL of Sample Buffer (marked "SAM BUF"). After reconstitution, it is recommended that controls be allowed to sit for 15 mins at room temperature and then mixed thoroughly but gently with a Vortex mixer. Reconstituted controls are stable for four weeks at -20°C. Controls should be not be repeatedly thawed, so controls should be appropriately aliquoted in appropriate volumes prior to being frozen.
4. Antibody HRP Conjugate
   Provided as ready to use.
5. Sample Buffer
   Provided as ready to use. Please shake before each use.
6. Dilution Buffer
   Provided as ready to use. Please shake before each use.
7. Wash Buffer (20X Concentrated)
   The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized water. Wash buffer is stable for 4 weeks at 2-8°C after dilution, so dilute only as needed.
8. Substrate Solution
   Provided as ready to use.
9. Stop Solution
   Provided as ready to use.

I.2. Dilution of samples and controls

1. Samples and controls need to be diluted with Sample Buffer for use with the assay. Please note that this section only applies to samples and controls, not standards. A sample dilution of 1:505 is generally suitable and should be performed as follows:

   a. Dilute 1:101 by mixing 5 µL of sample or control with 0.5 mL of Sample Buffer.
   b. Dilute another 1:5 by mixing 100 µL of prior mixture with 400 µL of Sample Buffer. Sample (or control) is accordingly diluted 1:505.

   Since IGFBP-3 levels can vary, dilution ratio may need to be adjusted as appropriate. Samples and controls must be used within 60 minutes once diluted.

I.3. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 50 µL of Dilution Buffer and 50 µL of diluted sample or 50 µL of standard or 50 µL of diluted control and mix well by repeated pipetting.
   Note: *A blank using 50 µL of Sample Buffer is recommended.*
2. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake at 350 rpm).
3. Aspirate well contents and wash five times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
4. Add 100 µL of the Antibody HRP Conjugate in each well.

5. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake at 350 rpm).
6. Aspirate well contents and wash five times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 µL of Substrate Solution in each well.
8. Incubate the plate for 30 mins in dark room at room temperature.
9. Stop the reaction by adding 100 µL of Stop Solution.
10. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.4. Determining the IGFBP-3 concentration

1. Using computer software, construct the IGFBP-3 calibration curve by plotting the mean change in absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding IGFBP-3 concentration on the X axis. A higher-grade polynomial, or four parametric logistic (4-PL) curve fit or non-linear regression are suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. IGFBP-3 concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. For diluted samples and controls, the values obtained must be multiplied by the dilution factor (ie. 505) to obtain the final IGFBP-3 concentration. The IGFBP-3 concentration is expressed in ng/mL.
   Note: *Samples with high IGFBP-3 concentrations (ie. fall above the range of the assay) should be further diluted with the Sample Buffer and rerun.*

J. Performance characteristics

J.1. Assay range

The IGFBP-3 ELISA Kit has an assay range from 0.4 – 30 ng/mL. The analytical sensitivity of the assay is 0.03 ng/mL.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

Warranty

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