V.2

IGFBP-6 ELISA
Kit Instructions

For the quantitative determination of IGFBP-6 in
human serum, plasma, fluids, and cell culture medium

Catalog #80595
96 Assays

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

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A. Intended Use

The IGFBP-6 ELISA kit is for the quantitative determination of IGFBP-6 human serum, plasma, fluids, and cell culture medium. 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-6 (Insulin-like Growth Factor Binding Protein-6) is part of the IGF-System with consists of six binding proteins (IGFBP1-6), IGF-I, and IGF-II. IGFBP-6 is 213 amino acids long with a mass of approximately 34 kDa, and unlike the other proteins, it has only three disulfide bridges on the C-terminal domain. As a result, IGFBP-6 has a significantly higher affinity (50 fold) for IGF-II vs IGF-I, and its suggested main function is to regulate the availability of IGF-II and therby influence cell differentiation, proliferation, migration, and survival.

C. Principle of the Assay

The IGFBP-6 ELISA kit is an ELISA sandwich assay for IGFBP-6. It utilizes two specific and high affinity antibodies for this protein. IGFBP-6 in the sample binds to the first antibody coated on the microtiter plate. In the following steps, a biotinylated anti-IGFBP-6 and streptavidin-peroxidase bind in-turn to the immobilized IGFBP-6. In the closing substrate reaction, the IGFBP-6 levels of the samples can be measured by color intensity.

D. Kit Storage

1. Upon receipt of the IGFBP-6 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.
3. Unless noted, all components are stable for 4 weeks at 2-8°C once opened.

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 (Lyophilized)	1 x 5 vials
CON1-2	Controls (Lyophilized)	1 x 2 vials
AB CONJ	Antibody Conjugate	1 x 12 mL
HRP	HRP Conjugate	1 x 12 mL
DIL BUF	Dilution Buffer	1 x 120 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	3x, adhesive

E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled or deionized water
Polypropylene microtubes
Vortex mixer
Microplate shaker (350 rpm)
Microplate reader (capable of reading 450 nm and ≥ 590 nm)

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 (10 μL), pipetting should be done as carefully as possible. A high quality 20 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.
6. Do not let the substrate or stop solution come in contact with metal parts including aluminum foil.

H. Sample Collection

Serum and plasma samples collected with EDTA or Heparin anticoagulant can be used. Hemolytic, icteric, and lipemic 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 1 year at -20°C. Avoid repeated freeze-thaw cycles of samples.

Other body fluids (breast milk, saliva, and amniotic fluid) and cell culture medium can be used.

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 0.1 ng/mL to 10 ng/mL. Dilute each standard with 0.75 mL of Dilution Buffer. 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. For reuse, thaw quickly at temperatures that do not exceed ambient. Standards are provided in the following concentrations: 0.1, 0.5, 1, 5, and 10 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 Dilution Buffer. 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. For reuse, thaw quickly at temperatures that do not exceed ambient.
4. Antibody Conjugate
   Provided as ready to use.
5. HRP Conjugate
   Provided as ready to use.
6. Dilution Buffer
   Provided as ready to 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

Samples and controls need to be diluted with Dilution Buffer for use with the assay. Please note that this section only applies to samples and controls, not standards. A sample dilution of 1:51 is generally suitable, and serum and plasma samples should be diluted at least 1:20. A 1:51 dilution should be performed as follows:

Dilute 1:51 by mixing 10 µL of sample or control with 500 µL of Dilution Buffer.

Since IGFBP-6 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. Remove desired number of microwell strips and store remainder.

1. In each well, add 100 µL of diluted sample, diluted control, or standard.
   Note: *A blank using 100 µL of Dilution 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 working 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 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 the HRP Conjugate in each well.
8. Cover the wells with sealing tape and incubate the plate for 30 minutes at room temperature (shake at 350 rpm).
9. 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.
10. Add 100 µL of Substrate Solution in each well.
11. Incubate the plate for 30 mins in dark room at room temperature.
12. Stop the reaction by adding 100 µL of Stop Solution.
13. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract $A_{\geq 590}$ values).

I.4. Determining the IGFBP-6 concentration

1. Using computer software, construct the IGFBP-6 calibration curve by plotting the mean change in absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding IGFBP-6 concentration on the X axis. A four parametric logistic (4-PL) curve fit is suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. IGFBP-6 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. 51) to obtain the final IGFBP-6 concentration. The IGFBP-6 concentration is expressed in ng/mL.
   Note: *Samples with high IGFBP-6 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-6 ELISA Kit has an assay range from 0.1 – 10 ng/mL.

J.2. Precision

The assay has a within-run and total precision of CV < 10% and analytical sensitivity of 0.03 ng/mL.

Warranty

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