Catalog #80577

V.4

Crystal Chem
High Performance Assays

IGFBP-2 ELISA
Kit Instructions

For the quantitative determination of IGFBP-2 in
human serum, plasma, fluids,
saliva, and cell culture medium

Catalog #80577
96 Assays

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

Crystal Chem, Inc.
955 Busse Road
Elk Grove Village, IL 60007, USA
Tel: (630) 889-9003 Fax: (630) 889-9021
E-mail: sales@crystalchem.com
Order online: www.crystalchem.com

Catalog #80577

TABLE OF CONTENTS

A. Intended Use

The IGFBP-2 ELISA kit is for the quantitative determination IGFBP-2 in human serum, plasma, fluids, saliva, 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-2 (Insulin-like Growth Factor Binding Protein-2) is an unglycosylated polypeptide of 31.3 kDa and the second most abundant IGFBP in the blood. At a cellular level, it seems to stimulate the growth of solid tumors, and as a result, has been correlated with certain types of tumors. It has also been used as a marker in for trisomy 18, physiological function, and growth disorders.

C. Principle of the Assay

The IGFBP-2 ELISA kit is an ELISA sandwich assay for IGFBP-2. It utilizes two specific, and high affinity, antibodies for this protein. IGFBP-2 in the sample binds to the first antibody coated on the microtiter plate. In the following step, the specific anti-IGFBP-2-Antibody bind, in turn, to the immobilized IGFBP-2. The second antibody is biotinylated and reacts with a streptavidin-HRP conjugate. In the closing substrate reaction, the IGFBP-2 levels of the samples can be measured by color intensity.

D. Kit Storage

1. Upon receipt of the IGFBP-2 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 POD-Conjugate	1 x 12 mL
DIL	Dilution Buffer	1 x 50 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
Standard laboratory glassware for buffer and reagent preparation
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. Each calibrator and sample should be assayed in duplicate.
2. Given the 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.
3. 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.
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 following standard venipuncture techniques can be used. Hemolytic samples should be avoided. Blood has to be allowed to clot, and then the clot must be removed by centrifugation. Samples should be chilled as soon as possible after sample withdrawal. For long-term storage, samples can be stored for up to 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 2 ng/mL to 80 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 two months 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: 2, 10, 20, 40, and 80

ng/mL.

3. Controls 1-2
   Controls are provided in lyophilized form with target value and ranges included on their labels. Dilute controls with 100 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 two months 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 POD-Conjugate
   Provided as ready to use.
5. Dilution Buffer
   Provided as ready to use. Please shake before each use.
6. 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.
7. Substrate Solution
   Provided as ready to use. *Photosensitive.*
8. Stop Solution
   Provided as ready to use.

I.2. Dilution of samples

Samples need to be diluted with Dilution Buffer for use with the assay. A sample dilution of 1:10-30 is generally suitable, but since IGFBP-2 levels can vary, particularly in different body fluids, dilution ratios may need to be adjusted as appropriate. An example 1:21 dilution should be performed as follows:

Dilute 1:21 by mixing 10 µL of sample with 200 µL of Dilution Buffer.

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. *Avoid foaming.*

1. In each well, add 100 µL of diluted sample or 100 µL of standard or 100 µL of control.
   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 1X 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-POD Conjugate in each well.
5. Incubate the plate for 30 minutes at room temperature (shake at 350 rpm).
6. Aspirate well contents and wash five times using 300 µL of 1X 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 15 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-2 concentration

1. Using computer software, construct the IGFBP-2 calibration curve by plotting the mean change in absorbance value for each standard (incl. blank) on the Y axis versus the corresponding IGFBP-2 concentration on the X axis. A higher-grade polynomial, 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-2 concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. For diluted samples, the values obtained must be multiplied by the dilution factor (ie. 21) to obtain the final IGFBP-2 concentration. The IGFBP-2 concentration is expressed in ng/mL.
   Note: *Samples with high IGFBP-2 concentrations (ie. fall above the range of the assay) should be further diluted with the Dilution Buffer and rerun.*

J. Performance characteristics

J.1. Assay range

The IGFBP-2 ELISA Kit has an assay range from 2 – 80 ng/mL. The analytical sensitivity of the assay is 0.2 ng/mL.

J.2. Precision

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

J.3. Cross reactivity

Does not cross-react with IGFBP-1 or IGFBP-3.

Warranty

Crystal Chem Inc. makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Buyer assumes all risk and liability resulting from the use of this product.

THERE IS NO WARRANTY OF MERCHANTABILITY OF THE PRODUCTS, OR THAT SUCH PRODUCTS ARE FIT FOR ANY PARTICULAR PURPOSE. CRYSTAL CHEM INC'S LIABILITY SHALL NOT EXCEED THE RETURN OF THE PURCHASE PRICE, AND UNDER NO CIRCUMSTANCES SHALL CRYSTAL CHEM INC. BE LIABLE FOR SPECIAL OR CONSEQUENTIAL DAMAGES, OR EXPENSES ARISING DIRECTLY OR INDIRECTLY FROM THE USE OF THIS PRODUCT.