Catalog #80578

V.3M

Crystal Chem

Rat IGFBP-2 ELISA Kit Instructions

For the quantitative determination of IGFBP-2 in rat serum

Catalog #80578
96 Assays

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

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A. Intended Use

The Rat IGFBP-2 ELISA kit is for the quantitative determination of IGFBP-2 in rat serum. 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 the second most abundant IGFBP in circulation. At a cellular level, IGFBP-2 seems to stimulate the proliferation and dissemination of solid tumors via an IGF-independent mechanism.

C. Principle of the Assay

The Rat IGFBP-2 ELISA kit is an ELISA sandwich assay for rat IGFBP-2. It utilizes two specific and high affinity antibodies for this protein.

D. Kit Storage

1. Upon receipt of the Rat 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
STD1-7	Standards	1 x 7 vials
DIL	Dilution Buffer	1 x 120 mL
CON	Control	1 vial
AB CONJ	Antibody Conjugate	120 µL
ENZ CONJ	Enzyme Conjugate	120 µL
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

Rat serum samples can be used. Store undiluted samples frozen in a tightly closed plastic vial until measurement. 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 1-7
   Standards are provided in lyophilized form with concentrations ranging from 0.03125 ng/mL to 2 ng/mL. Dilute each standard with 1 mL of Dilution Buffer (marked “DIL”). 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 2 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: 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, and 2 ng/mL.
3. Dilution Buffer
   Provided as ready to use.
4. Control
   Control is provided in lyophilized form with target value and range included on the label. Reconstitute control with 250 µL of Dilution Buffer. After reconstitution, it is recommended that control be allowed to sit for 15 mins at room temperature and then mixed thoroughly but gently with a Vortex mixer. Reconstituted control is stable for 2 months at -20°C.
5. Antibody Conjugate
   The 120 µL of Antibody Conjugate is provided as 100X Concentrated. It should be diluted immediately before use 1:100 with Dilution Buffer.

6. Enzyme Conjugate
   The 120 µL of Enzyme Conjugate is provided as 100X Concentrated. It should be diluted immediately before use 1:100 with Dilution Buffer.
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 reconstituted 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 between 1:20 – 1:500 will be needed depending on the sample. A dilution of 1:100 is generally suitable and should be performed as follows:

   a. Dilute 1:100 by mixing 5 µL of sample or control with 495 µL of Dilution Buffer.

   Since rat IGFBP-2 levels can vary, dilution ratio may need to be adjusted as appropriate. Samples should 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 as instructed immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 100 µL of diluted sample or 100 µL of standard or 100 µL of diluted control and mix well by repeated pipetting.
   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 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 diluted 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 diluted Enzyme Conjugate in each well.
8. Cover the wells with sealing tape and incubate the plate for 30 mins 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_{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 calibrator (incl. blank) on the Y axis versus the corresponding IGFBP-2 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. Rat IGFBP-2 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, 100) to obtain the final IGFBP-2 concentration. The IGFBP-2 concentration is expressed in ng/mL.
   Note: *Samples with high rat 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 Rat IGFBP-2 ELISA Kit has an assay range from 0.03125 – 2 ng/mL. The analytical sensitivity of the assay is 0.01 ng/mL.

J.2. Precision

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

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