Human IGFBP1 ELISA Kit

Enzyme Immunoassay for the quantification of IGFBP1 in Human serum.

Catalog number: ARG82798

Package: 96 wells

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

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INTRODUCTION

Insulin-like growth factor-binding protein 1 (IBP-1) also known as placental protein 12 (PP12) is a protein that in humans is encoded by the IGFBP1 gene.

This gene is a member of the Insulin-like growth factor-binding protein (IGFBP) family and encodes a protein with an IGFBP domain and a type-I thyroglobulin domain. The protein binds both insulin-like growth factors (IGFs) I and II and circulates in the plasma. Binding of this protein prolongs the half-life of the IGFs and alters their interaction with cell surface receptors. Alternate transcriptional splice variants, encoding different isoforms, have been characterized. [Provide by Wikipedia: IGFBP1]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for IGFBP1 has been pre-coated onto a microtiter plate. Standards, Controls or samples are pipetted into the wells and any IGFBP1 present is bound by the immobilized antibody. After incubation, wash plate with Wash Buffer to remove unbound substance. Added antibody-conjugate specific for IGFBP1 to each well and incubate. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of IGFBP1 bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of IGFBP1 in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Standards A (0 µg/L)	2 mL (ready to use)	4°C
Standards B to F (1, 5, 30, 100, 250 µg/L)	0.5 mL each vial (ready to use)	4°C
Control 1 & 2	0.5 mL each vial (ready to use)	4°C
Diluent Buffer	26 mL (ready to use)	4°C
100X Antibody Conjugate (Anti-IGFBP1 Antibody conjugate HRP)	250 µL	4°C
10X Wash Buffer	50 mL	4°C
TMB substrate	16 mL (ready to use)	4°C (protect from light)
STOP solution	6 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- Unused wells must be stored at 2-8 °C in the sealed foil pouch and used in the frame provided.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.

- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Samples containing sodium azide should not be used in the assay.
4. Specimens should be capped and may be stored for up to 1 day at 2-8°C prior to assaying. Specimens stored for a longer time (up to 3 months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 mL of 10X Wash Buffer into 450 mL of distilled water to a final volume of 500 mL) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.
- 1X Antibody Conjugate: Dilute 1:100 in Diluent Buffer before use (E.g., 20 µL of 100X Antibody Conjugate in 2 mL of Diluent Buffer). Do not store diluted Antibody Conjugate.

ASSAY PROCEDURE

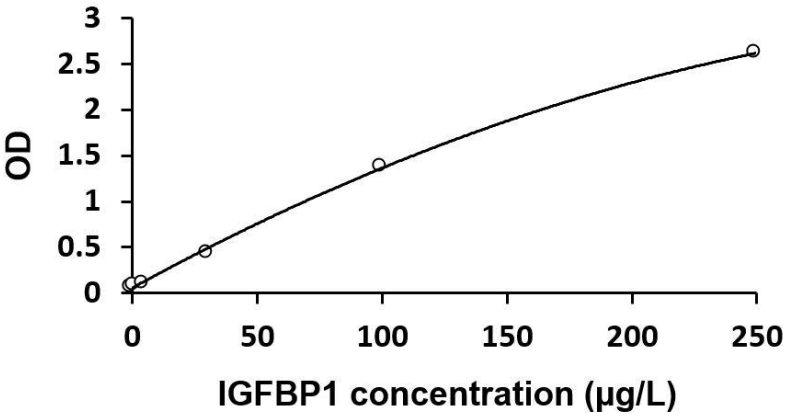
All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add **25 µL** of **samples, Controls and Standards** to the **Antibody Coated microplate**.
2. Add **100 µL** of the **Diluent Buffer** to each well.
3. Incubate at **RT** for **30 minutes** on a microplate shaker.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with **1× Wash Buffer (300 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.
5. Add **100 µL** of the **1X Antibody Conjugate** to each well.
6. Incubate at **RT** for **30 minutes** on a microplate shaker.
7. Aspirate each well and **wash as step 4**.
8. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate for **10-15 minutes** at room temperature in the dark.
9. Immediately Add **50 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at **450 nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 20 minutes** after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human IGFBP1 ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

Standard	Optical Density (450 nm)	Value (µg/L)
Standard A	0.076	0
Standard B	0.087	1
Standard C	0.123	5
Standard D	0.456	30
Standard E	1.380	100
Standard F	2.615	250
Unknown	0.119	4.5



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and samples.
2. Subtract the mean absorbance value of the Standard A from the mean absorbance values of the Standards, controls and serum samples.
3. Using log-log, semi-log or 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.
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. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If a sample reads more than 220 µg/L, then dilute it with Standard A at a dilution of no more than 1:10. The result obtained should be multiplied by the dilution factor.

QUALITY ASSURANCE

Sensitivity

0.5 µg/L

Cross Reactivity

Substance	Concentration Range (µg/L)	Apparent IGFBP-1 value (µg/L)
IGFBP-2	Up to 5000	Not Detected
IGFBP-3	Up to 10000	Not Detected
IGFBP-4	Up to 5000	Not Detected
IGFBP-5	Up to 5000	Not Detected

Intra-assay and Inter-assay precision

The CV value of intra-assay was 2.4-3.4% and inter-assay precision was 4.9-7.4%

Recovery

Spiked samples were prepared by adding defined amounts of IGFBP-1 to three patient serum samples (1:1). The results (in µg/L) are tabulated below:

Sample	Result	Expected Result	Recovery %
1 Unspiked	5.0	-	-
+6.5	5.8	5.75	100.9
+35	20	20	100.0
+174	90	89.5	100.6
2 Unspiked	20	-	-
+6.5	14	13.3	105.3
+35	29	24.5	118.4
+174	100	97	103.1
3 Unspiked	110	-	-
+6.5	62	58.3	106.3
+35	80	72.5	110.3
+174	155	133	116.5