Human IGF1 ELISA Kit

Enzyme Immunoassay for the quantification of Insulin-like Growth Factor I (IGF1) in serum and plasma

Catalog number: ARG80495

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 622 1320

Fax: +886 (3) 553 0266

Email: info@arigobio.com

INTRODUCTION

Insulin-like growth factors (IGF) I and II play a pivotal role in regulating the proliferation, differentiation and specific functions of many cell types. IGF-I is identical with Somatomedin C (Sm-C) and has a molecular weight of 7649 Daltons. Its major regulators are growth hormone (GH) and nutrition, although its production in specific tissues is affected by a multitude of tropic hormones and other peptide growth factors. In contrast to many other peptide hormones, IGFs are avidly bound to specific binding proteins (IGFBP). The seven classes of IGFBPs which are known at present either bind IGF-I and IGF-II with similar affinities or show a preference for IGF-II.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Human IGF-I has been pre-coated onto a microtiter plate. IGF-I in samples will be release by diluted with an acidic Sample/Standard diluent buffer from IGF1BPs. Diluted standards or samples are pipetted into the wells and any IGF-I present is bound by the immobilized antibody. Then a biotin-conjugated antibody specific for IGF-I is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of IGF-I bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of IGF-I

in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate (8x12) wells	4°C.
Standard 1-5 (2, 5, 15, 30, 50 ng/ml)	5 vials (lyophilized)	4°C. Stored at 20 °C after reconstitution.
Sample/Standard diluent buffer	25 ml (ready-to-use)	4°C
Control 1 (93 ng/ml; acc. range: 77-112 ng/ml)	1 vial (lyophilized)	4°C. Stored at 20 °C after reconstitution.
Control 2 (428 ng/ml; acc. range: 343-514 ng/ml)	1 vial (lyophilized)	4°C. Stored at 20 °C after reconstitution.
Biotin-conjugated Antibody	9 ml (ready-to-use)	4°C
HRP-Streptavidin solution	12 ml (ready-to-use)	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	12 ml (ready-to-use)	4°C (Protect from light)
STOP solution	12 ml (ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at ≥ 590 nm as reference wavelength)
- Pipettes and pipette tips
- Deionized or distilled water
- Microplate shaker (shaking amplitude 3 mm; approx. 350 rpm)
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times after receipt until its expiry date. The lyophilized reagents should be stored at 20 °C after reconstitution. Avoid repeated thawing and freezing.
- The shelf life of the components after initial opening is warranted for 4 weeks, store the unused strips and microtiter wells airtight together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided.
- The reconstituted components standards and Controls must be stored at –20°C for up to 4 weeks. For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing).
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Bring all reagents to room temperature (20- 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming. Reagents with different lot numbers cannot be mixed.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- When performing the assay, Blank, Standards, Controls and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, Biotin-conjugated

Antibody HRP-Streptavidin solution as well as the TMB Substrate Solution should be added to the plate in the same order and in the same time interval as the samples. STOP Solution should be added to the plate in the same order as TMB Substrate Solution.

- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time. Collect serum and assay immediately or aliquot and store samples at ≤ -20°C for up to 3 months. Samples should be frozen only once at-20 °C prior to assay. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C for up to 3 months. Samples should be frozen only once at-20 °C prior to assay. Avoid repeated freeze-thaw cycles.

Note:

1. EDTA and citrate plasma samples may give slightly lower results.
2. Prior to assay, thawed samples should be inverted several times prior to testing

3. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. The 1:20 diluted Washing Buffer is stable for up to 4 weeks at 2-8°C.
- **Controls:** Reconstitute each control with **0.5 ml** of **Sample/Standard diluent buffer**, keep reconstituted reagents at room temperature for 15 minutes and then gently mix (no foam should result) to dissolve completely. Store the reconstituted controls at –20°C for up to 4 weeks. *Dilute the controls at 1:21 with Sample/Standard diluent buffer before use.* (e.g. 200 µl of Sample/Standard diluent buffer + 10 µl control)
- **Samples**: Prior to use, *dilute the samples at 1:21 dilution with Sample/Standard diluent buffer,* mix thoroughly. (e.g. 200 µl Sample/Standard diluent buffer + 10 µl sample). Using 2 x 20 µl of this dilution in the assay.

  Note:

  1. Serum and plasma samples must be diluted at least 1:10 in Sample/Standard diluent buffer in order to achieve sufficient acidification of the samples.
  2. If the expected IGF-I values the samples is higher than the highest standard, the samples can be diluted higher in Sample/Standard diluent buffer.
  3. Diluted Samples can be stored at 20-25°C for up to 2 hours.

- **Standards**: Standard 1-5 (lyophilized). Reconstitute each standard with **0.5 ml** of **Sample/Standard diluent buffer** to generate standards of **2 ng/ml, 5 ng/ml, 15 ng/ml, 30 ng/ml and 50 ng/ml**. The Sample/Standard diluent buffer serves as zero standard (0 ng/ml). Keep reconstituted reagents at room temperature for 15 minutes and then gently mix (no foam should result) to dissolve completely.

Standard	0	1	2	3	4	5
ng/ml	0	2	5	15	30	50
nmol/L	0	0.26	0.66	1.96	3.92	6.54

ASSAY PROCEDURE

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

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **80 µl** of **Biotin-conjugated Antibody** into each well would like to be used in this assay.
3. Add **20 µl** of **standards, diluted controls, diluted samples** (dilute 1:21 with Sample/Standard diluent buffer) and **zero controls** (Sample/Standard diluent buffer) in duplicates into appropriate wells. Cover the plate and incubate for **1 h at room temperature** on a microplate shaker (~350rpm).
4. Aspirate each well and wash, repeating the process 4 times for a **total 5 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 **HRP-Streptavidin solution** to each well. Cover wells and incubate for **30 minutes at room temperature** on a microplate shaker (~350rpm).
6. Aspirate each well and **wash as step 4**.
7. Add **100 µl** of **TMB Substrate** reagent to each well. Incubate for **15 minutes at room temperature in dark**.
8. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at **450nm** immediately. (optional: read at ≥ 590 nm as reference wavelength) It is recommended reading the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear or log-log 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.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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.

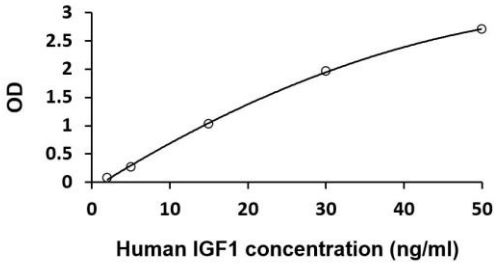
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. The IGF-I concentration in ng/mL of the samples can be calculated by multiplication with the respective dilution factor (If followed the protocol as above, the dilution factor would be 21).

(e.g. If the calculated IGF-1 concentration from diluted sample is 5 ng/ml. If the dilution factor (1:21) is taken into account the IGF-I concentration of the undiluted sample is

5 ng/ml × 21 = 105 ng/ml

EXAMPLE OF TYPICAL STANDARD CURVE



QUALITY ASSURANCE

Quality criteria

For the evaluation of the assay it is required that the absorbance values of the zero standard should be below 0.25, and the absorbance of standard 5 should be above 1.00.

Samples, which yield higher absorbance values than Standard 5, should be re-tested with a higher dilution.

Sensitivity

The minimum detectable dose (MDD) of IGF-I ranged from 2-50 ng/ml. The mean MDD was 0.091 ng/ml.

Specificity

No significant cross-reactivity or interference with the following factors was observed (<0.1%):

IGF-II, Insulin, C-Peptide

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5.81% and inter-assay precision was 8.57%.

Recovery

85-102%