Mouse / Rat IGFI ELISA Kit

Enzyme Immunoassay for the quantification of Mouse and Rat IGFI in serum and plasma (EDTA, heparin, citrate).

Catalog number: ARG80702

Package: 96 wells

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INTRODUCTION

Insulin-like growth factor 1 (IGF-1), also called somatomedin C, is a hormone similar in molecular structure to insulin which plays an important role in childhood growth, and has anabolic effects in adults.

IGF-1 is a protein that in humans is encoded by the IGF1 gene. IGF-1 consists of 70 amino acids in a single chain with three intramolecular disulfide bridges. IGF-1 has a molecular weight of 7,649 Daltons.

IGF-1 is produced primarily by the liver. Production is stimulated by growth hormone (GH). Most of IGF-1 is bound to one of 6 binding proteins (IGF-BP). IGFBP-1 is regulated by insulin. IGF-1 is produced throughout life; the highest rates of IGF-1 production occur during the pubertal growth spurt. The lowest levels occur in infancy and old age.

A synthetic analog of IGF-1, mecasermin, is used in children for the treatment of growth failure. [Provide by Wikipedia: Insulin-like growth factor 1]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for IGF-I has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IGF-I present is bound by the immobilized antibody. After washing away any unbound substances, an Antibody Conjugate specific for IGF-I is added to each well and incubate. After washing away any unbound antibody, a HRP Conjugate added to the wells. After washing away any unbound substances, the TMB Substrate 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 STOP solution and the intensity of the color is measured at a wavelength of 450nm. The concentration of IGF-I in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, aliquot and store lyophilized reagents at -20°C after reconstitution and avoid freeze/thaw. 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 1-5 (0.5 / 2.5 / 6 / 12 / 18 ng/ml)	5 vials	4°C
Control 1 (Control Serum 1, lyophilized, range: 228-343 ng/ml)	1 vial	4°C
Control 2 (Control Serum 2, lyophilized, range: 498-748 ng/ml)	1 vial	4°C
Diluent Buffer	125 ml (ready to use)	4°C
Antibody Conjugate (Biotinylated Anti-m/rIGF-I Antibody)	7 ml (ready to use)	4°C
Streptavidin-HRP Conjugate	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
Sealing Tape	2 ea	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
- 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 unused strips and microtiter wells airtight together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided.
- Store the kit at 2-8°C at all times.
- The lyophilized reagents after reconstitution should be stored at-20°C.
- The Stock Standard and Control 1 & 2 should be aliquoted into smaller portions before use to ensure product integrity and store the aliquoted Stock Standard at-20°C. Avoid repeated freeze-thaw cycles.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X 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.
- Use a new adhesive plate cover for each incubation step.
- 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: Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

1. Sample dilution: Dilute 1:100 with Diluent Buffer (recommend), mix immediately, incubate at room temperature for 15 mins, max 2 hours.
2. Serum and Plasma samples must be diluted at least 1:10 in Diluent Buffer in order to achieve sufficient acidification of the samples.
3. The assay required sample volume is 10 µL, min. 5 µL.
4. Cell culture medium is suitable as sample matrix after pre-dilution of 1:2 with Diluent Buffer.
5. Do not use haemolytic, icteric or lipaemic specimens.
6. Samples containing sodium azide should not be used in the assay.
7. Aliquot samples for testing and store at-80°C. Avoid repeated freeze-thaw cycles. Perform dilutions in Diluent Buffer as necessary.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 25 ml of 20X Wash Buffer into 475 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.
- Control 1 and 2: Reconstituted with 500 µL of the Diluent Buffer. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer. After reconstitution dilute the Control 1 and 2 with the Diluent Buffer in the same ratio as the sample. The reconstituted reagent is stable for up to 2 months at-20°C.

- **Standards (Recombinant IGF-I Standards):** Reconstituted with 1 ml of the Diluent Buffer. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer. The reconstituted reagent is stable for up to 2 months at-20°C.

Standards	A	B	C	D	E
ng/ml	0.5	2.5	6	12	18

ASSAY PROCEDURE

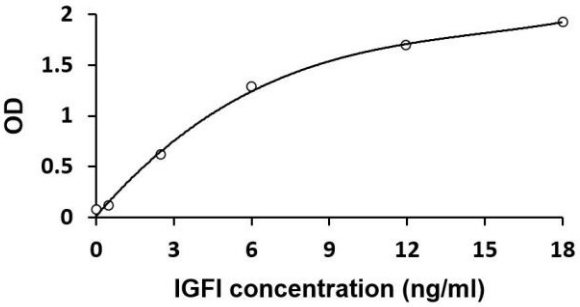
All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates. When performing the assay, Blank, Standards, Controls 1 and 2 and the samples should be pipette as fast as possible (e.g. <15 minutes).

1. Add **50 µL** of **samples, Control 1 and 2 or Standards** to the Antibody-coated microplate.
2. Add **50 µL** of **Antibody Conjugate** to all wells used.
3. Incubate at **RT** for at least **1 hour** on a microplate shaker (350 rpm).
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 the **Streptavidin-HRP Conjugate** to each well. Incubate at **RT** for **30 mins** on a microplate shaker (350 rpm).

6. Aspirate each well and wash as step 4.
7. Warm TMB substrate to RT. Add 100 µL of TMB Substrate to each well, including the blank wells. Incubate for 30 minutes at room temperature in the dark.
8. Add 100 µL of Stop Solution to each well, including the blank wells. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately. It is recommended reading the absorbance within 30 minutes.

EXAMPLE OF TYPICAL STANDARD CURVE

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



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. 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.
3. Use 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 the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

0.315 ng/ml

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay precision was ≤ 10%.