Human IGF-I ELISA Kit

Enzyme Immunoassay for the quantification of Insulin-like Growth Factor I (IGF-1) in serum and plasma

Catalog number: ARG80495

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

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	5 vials (lyophilized)	4°C.
Sample/Standard diluent buffer	25ml (ready-to-use)	4°C
Control 1	1 vial (lyophilized)	4°C
Control 2	1 vial (lyophilized)	4°C
Biotin-conjugated Antibody	9 ml (ready-to-use)	4°C
HRP-Streptavidin solution	12 ml (ready-to-use)	4°C (Protect from light)
20X Wash buffer	50ml	4°C
TMB substrate	12ml (ready-to-use)	4°C (Protect from light)
STOP solution	12ml (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.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All materials should be equilibrated to room temperature (RT) before use.

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. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. 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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- Controls: Reconstitute each control with 0.5ml Sample/Standard diluent buffer, keep reconstituted reagents at room temperature for 15 minutes and then gently mix to dissolve completely. Dilution the controls 1:21 with Sample/Standard diluent buffer before use.
- Samples: Dilution the samples 1:21 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 up to 2 hours.
- Standards: Standard 1-5 (lyophilized). Reconstitute each standard with 0.5ml Sample/Standard diluent buffer to generate standards of 2ng/ml, 5ng/ml, 15ng/ml, 30ng/ml and 50ng/ml. The Sample/Standard diluent buffer serves as zero standard (0 ng/ml)

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 80µl of Biotin-conjugated Antibody into each well.
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. Incubate for 1 h at room temperature on 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 (300µl) 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 microplate shaker (~350rpm).
6. Aspirate each well and wash as step 3.
7. Add 100µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature in dark.
8. Add 100µl 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)

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.

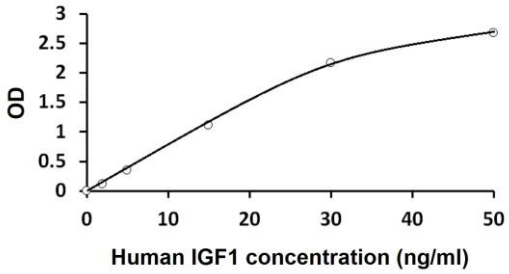
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. 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 0.09 ng/ml.

Specificity

No significant cross-reactivity or interference with the following factors was observed:

IGF-II, Insulin, C-Peptide

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5.8% and inter-assay precision was 6.17%.

Recovery

85-102%

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063

Phone: 617-419-2019 ● FAX: 617-419-1110

www.EagleBio.com ● info@eaglebio.com

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.