Innovative RESEARCH Results Driven Research

Human Insulin-like Growth Factor 1 (IGF-1) ELISA Kit

Introduction

Insulin-like Growth Factor 1 (IGF-1) is a 70 amino acid polypeptide protein hormone with molecular mass of 7.65 kDa (1). IGF-1 is produced primarily by the liver in response to the stimulation of growth hormone. It is transported in plasma bound to different forms of IGF-1 binding proteins (2). It also binds to specific IGF-1 tyrosine kinase receptor and the insulin receptor. Inhibition IFG-1 receptor reduces pancreatic cancer growth and angiogenesis (3). IGF-I regulates cellular proliferation, differentiation, apoptosis, and amyloid precursor protein family (4 - 5). It may be important in the pathophysiological processes underlying chronic disease, including type 2 diabetes mellitus, coronary heart disease, cancer, and Alzheimer's disease (6 - 8). Increased levels of IGF lead to an increased risk of cancer (9). IGF-I stimulates osteoblast proliferation, bone formation, and increases bone volume (10). It is a potent neurotrophic as well as a neuroprotective factor found in the central and the peripheral nervous systems of the brain (11).

Principal of the Assay

The Human IGF-1 ELISA kit is designed for detection of human IGF-1 in plasma, serum and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique, which measures IGF-1 in less than 5 hours. A monoclonal antibody specific for human IGF-1 has been pre-coated onto a microplate. Human IGF-1 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for human IGF-1, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-antibody, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Human IGF-1 Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human IGF-1.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Human IGF-1 Standard:** Recombinant human IGF-1 in a buffered protein base (96 ng, lyophilized).
- **Biotinylated IGF-1 Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against IGF-1 (140 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Plasma/Serum Pretreatment Buffer (1x)**: A ready to use plasma/serum pretreatment buffer (15 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store components of the kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20^{0}C
- Store Microplate, Pretreatment Buffer, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8^{0}C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8^{0}C.
- Store Standard at 2-8^{0}C before reconstituting with Diluent and at -20^{0}C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Pretreat plasma sample as follows: Add 20 µl of plasma sample into 60 µl of Plasma/Serum Pretreatment Buffer (1:4 dilutions) and incubate for 10 minutes at room temperature . Dilute pretreated plasma sample 1:10 into MIX Diluent and assay. The final dilution factor is 40x. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and pretreat serum sample as follows: Add 20 µl of serum sample into 60 µl of Plasma/Serum Pretreatment Buffer (1:4 dilutions) and incubate for 10 minutes at room temperature. Dilute pretreated serum sample 1:10 into MIX Diluent and assay. The final dilution factor is 40x. Store samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.

- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x)**: If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2 - 8^{0}C.
- **IGF-1 Standard:** Reconstitute the 96 ng of human IGF-1 Standard with 4 ml of MIX Diluent to generate a stock solution of 24 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the Standard solution (24 ng/ml) 1:2 with equal volume of MIX Diluent to produce 12, 6, 3, 1.5 and 0.75 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[H.IGF-1] (ng/ml)
P1	1 part Standard (24 ng/ml)	24.00
P2	1 part P1 + 1 part MIX Diluent	12.00
P3	1 part P2 + 1 part MIX Diluent	6.00
P4	1 part P3 + 1 part MIX Diluent	3.00
P5	1 part P4 + 1 part MIX Diluent	1.50
P6	1 part P5 + 1 part MIX Diluent	0.75
P7	MIX Diluent	0.00

- **Biotinylated IGF-1 Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20^{0}C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1: 20 with reagent grade water.
- **Streptavidin-Peroxidase Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20^{0}C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well, and cover wells and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated IGF-1 Antibody to each well and incubate for two hours.
- Wash the microplate as described above.

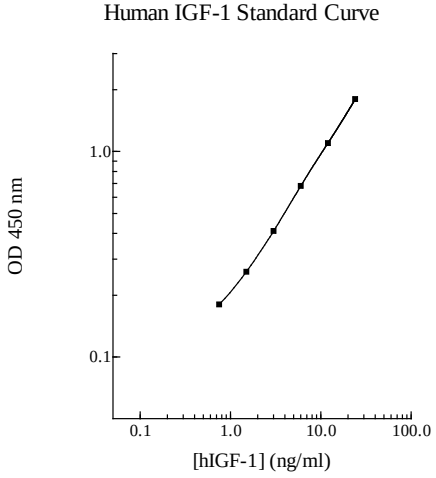
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis and draw a best-fit curve through the points on the graph. Plotting the log-log graph may linearize the data and the best-fit line can be determined by regression analysis of the linear portion of the curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of IGF-1 is typically 0.7 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.2% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:20	92%	94%
1:40	99%	100%
1:80	103%	104%

Recovery

Standard Added Value	1 – 10 ng/ml
Recovery %	87-109 %
Average Recovery %	98.5 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Monkey	None
Mouse	None
Rat	None
Swine	None
Rabbit	None
Human	100%

References

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