Human IGF1 ELISA Kit

Enzyme Immunoassay for the quantification of Insulin-like growth factor I in human serum.

Catalog number: ARG80491

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PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification IGF-I in serum.

This assay employs the competitive quantitative enzyme immunoassay technique. Patient samples, standards and controls are first acidified and neutralized.

A monoclonal antibody directed towards antigenic site of IGF-I has been pre-coated onto a microtiter plate. The pre-treated sample is incubated at room temperature with biotinylated IGF-I. The wells are washed and incubated with Streptavidin-HRP complex. After washing away any unbound antibody conjugate, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of IGF-I present in the samples. The color development is stopped by the addition of STOP solution 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
Anti IGF-I monoclonal antibody coated microplate	12 strips X 8 wells	4°C
40X Wash Buffer	30ml	4°C
Biotinylated IGF-I	14ml (Ready-to-use)	4°C
Streptavidin-HRP complex	20ml (Ready-to-use)	4°C

TMB substrate	14ml (Ready-to-use)	4°C (Protect from light)
STOP solution	14ml (Ready-to-use)	4°C
Standard 0-5	1ml each (Ready-to-use)	4°C
0.2M HCl	2 X 3ml (Ready-to-use)	4°C
Neutralization buffer	3ml (Ready-to-use)	4°C
Control Low (29.91 ng/ml, accept. range: 16.45-43.37 ng/ml)	1ml (Ready-to-use)	4°C
Control High (276.2 ng/ml, accept. range: 151.9-400.5 ng/ml)	1ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 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 40X 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.

SAMPLE COLLECTION & STORAGE INFORMATION

Repeated freezing and thawing of samples should be avoided.

Serum: Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at RT. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require a longer clotting time. Assay immediately, or store at 2-8 °C up to 24hrs. For longer storage, aliquot and store samples at ≤ -20 °C up to 12 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. Storage: up to 2 weeks at RT.
- Sample dilution: In the initial assay, if a specimen is found to contain more than the highest standard, the specimens can be diluted with Standard 0 and re-assay or reported as >600 ng/ml. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

ASSAY PROCEDURE

Acidification and neutralization

1. Pipette 50 µl of standards, controls and samples into the 1.5ml reaction caps.
2. Add 50 µl 0.2M HCl into all tubes.
3. Mix thoroughly and incubate for 30 mins at RT.

4. Add 10 µl Neutralization buffer to all caps and mix the solution. (It is not necessary to check the pH at this step)
5. Continue with ELISA assay within 10 minutes.

IGF-I ELISA procedure

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 20 µl of the pre-treated standards, controls and samples into the appropriate wells of Microtiter Strips.
3. Add 100 µl of Biotinylated IGF-I into wells.
4. Mix for 10 seconds and incubate at 2 hours at RT.
5. Remove the foil and discard. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (400 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.
6. Add 150 µl of Streptavidin-HRP complex conjugate into wells.
7. Incubate for 30 mins at RT.
8. Aspirate each well and wash as step 5.
9. Add 100 µl of TMB substrate solution into each well. Incubate for 15 mins at RT. Avoid exposure to light.
10. Add 100 µl of Stop Solution to each well and shake lightly to ensure homogeneous mixing.
11. Read the OD with a microplate reader at 450nm (with a reference wavelength between 620nm and 650nm) within 10 minutes.

CALCULATION OF RESULTS

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

2. Using 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.

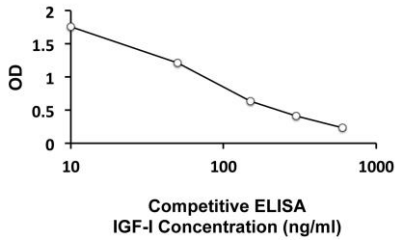
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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 diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

9.75 ng/ml

Assay Range

10-600 ng/ml

Specificity

No significant cross-reactivity was found for the following factors:

IGF-II (1.02%), Insulin (3.3%)

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6.4-7.4% and CV value of inter-assay precision was 10.3-14.8%.

Recovery

86.1-126.4

Linearity

85.4-112.7%