FOR RESEARCH USE ONLY!

IGF-I (human) ELISA Kit

rev 06/15

(Catalog # K4775-100, 100 assays; Store at -20°C)

I. Introduction:

BioVision's human IGF-I ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IGF-I. This assay employs an antibody (Ab) specific for human IGF-I coated on a 96-well plate. Standards and samples are pipetted into the wells and IGF-I present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IGF-I detection antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IGF-I bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. This ELISA kit only can detect a free form of human IGF-I & shows no cross-reactivity with any of the cytokines tested e.g., human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN-γ, Leptin (OB), MCP-1, MCP-3, MDC, MIP-1α, MIP-1 β, MIP-1δ, PARC, RANTES, SCF, TARC, TGF-β, TIMP-1, TIMP-2, TNF-α, TNF-β, TPO, VEGF. The minimum detectable dose of IGF-I is typically less than 0.1 ng/ml. Detection Range: 0.1 ng/ml - 30 ng/ml. The intra-Assay reproducibility is CV<10% & inter-Assay is CV<12%.

II. Application:

Quantitative protein detection, establishing normal range, validation of antibody array results.

III. Specificity:

The antibody pair provided in this kit recognizes human IGF-I.

IV. Sample Type:

- Serum & plasma
- Cell culture supernatants
- Urine

V. Kit Contents:

Components	K4775-100	Part No.
IGF-I Ab-coated Microplate (Item A), 96 wells	12 stripsx8 wells	K4775-100-1
Wash Buffer (20x) (Item B)	25 ml	K4775-100-2
Human IGF-I Standard (Item C)	2 vials	K4775-100-3
Assay Diluent C (Item L)	2x30 ml	K4775-100-4
Detection Antibody (Item F)	2 vial	K4775-100-5
HRP-Streptavidin (120x) (Item G)	200 µl	K4775-100-6
TMB (Item H)	12 ml	K4775-100-7
Stop Solution (Item I)	8 ml	K4775-100-8

VI. User Supplied Reagents and Equipment:

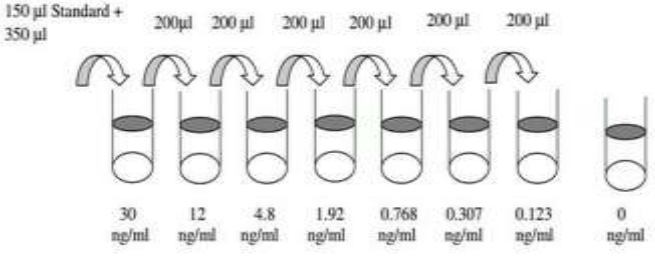
- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Distilled or deionized water.

VII. Storage and Handling:

May be stored for up to 6 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at –80°C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. **Note:** the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

VIII. Reagent Preparation:

- Bring all reagents and samples to room temperature (18 - 25°C) before use.
- Sample dilution: If your samples need to be diluted, Assay Diluent C (Item L) should be used for dilution of serum/plasma/culture supernatants & urine. Suggested dilution for normal serum/plasma: 2-20 fold*.
  *Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.
- Preparation of standard: Briefly spin the vial of Item C then add 400 µl Assay Diluent C into Item C vial to prepare a 100 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 150 µl IGF-I standard from the vial of Item C, into a tube with 350 µl Assay Diluent C to prepare a 30 ng/ml standard solution. Pipette 300 µl Assay Diluent C into each tube. Use the stock standard solution to produce a dilution series (shown). Mix each tube thoroughly before the next transfer. Assay Diluent C serves as the zero standard (0 ng/ml).



- If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

- Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of Assay Diluent C into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with Assay Diluent C and used in step 4 of Assay Protocol.
- Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 120-fold with Assay Diluent C.

IX. Assay Protocol:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of 1x prepared biotinylated detection antibody (see Reagent Preparation) to each well. Incubate for 1 hour at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
5. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation) to each well. Incubate for 45 minutes at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of TMB (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
7. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.
8. Calculation**:** Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

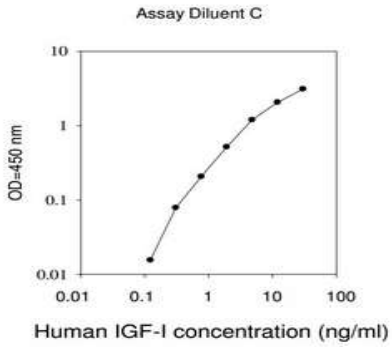


Figure 1: Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

Sample Type	Average % Recovery	Range (%)
Serum	119.6	105-138
Plasma	105.4	81-121
Cell culture media	102.5	98-110

Table 1: Recovery: Recovery was determined by spiking various levels of IGF-I into human serum, plasma and cell culture media.

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	116.4	111.9	103.3
	Range (%)	108-124	107-125	94-118
1:4	Average % of Expected	121.9	104.6	84.62
	Range (%)	114-130	81-120	77-92

Table 2: Linearity

X. RELATED PRODUCTS:

IGF-1, human recombinant (4119)
IGF-1, murine recombinant (4120)
IGF-1, rat recombinant (4121)
R3 IGF-1, human recombinant (4216)
Anti-Rat IGF-1 Antibody (5121)
IGF-1 Antibody (5119, 5120R)
IGF-I sR (human) ELISA Kit (K4776)

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