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Product Information

Human IGF-I ELISA Kit
for serum, plasma, cell culture supernatant, and urine

Catalog Number **RAB0228**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The 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 in serum, plasma, cell culture supernatants, and urine. This kit can only detect the free form of human IGF-I. This assay employs an antibody 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 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.

Components

1. Human IGF-I Antibody-coated ELISA Plate (Item A) - RAB0228A: 96 wells (12 strips × 8 wells) coated with anti-human IGF-I.
2. 20x Wash Buffer (Item B) - RABWASH4: 25 mL of 20x concentrated solution.
3. Lyophilized Human IGF-I Protein Standard (Item C) - RAB0228C: 2 vials, recombinant human IGF-I.
4. ELISA 1x Assay/Sample Diluent Buffer C (Item L) - RABELADC: 2 bottles of 30 mL diluent buffer
5. Biotinylated Human IGF-I Detection Antibody (Item F) - RAB0228D: 2 vials of biotinylated anti-human IGF-I (each vial is enough to assay half microplate).
6. HRP-Streptavidin (Item G) - RABHRP5: 200 µL of 120x concentrated HRP-conjugated streptavidin.
7. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3: 12 mL of 3,3′,5,5′-tetra-methylbenzidine (TMB) in buffered solution.
8. ELISA Stop Solution (Item I) - RABSTOP3: 8 mL of 0.2 M sulfuric acid.

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µL to 1 mL volumes
3. Adjustable 1-25 mL pipettes for reagent preparation
4. 100 mL and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. SigmaPlot software (or other software which can perform four-parameter logistic regression models)
8. Tubes to prepare standard or sample dilutions

Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

1. Bring all reagents and samples to room temperature (18–25 °C) before use.

2. No dilution is needed for Assay Diluent C (Item L). This reagent is provided at 1x concentration. Move on to step 3.

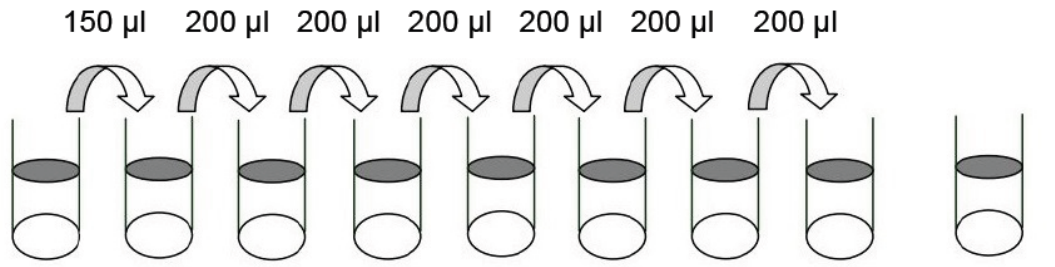
3. Sample dilution: 1x Assay Diluent C (Item L) should be used for dilution of serum/plasma/culture supernatants/urine. The suggested dilution for normal serum/plasma is 2 to 20-fold.

   Note: Levels of IGF-I may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of standard: **Briefly spin a vial of Item C**. Add 400 µL of 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 of IGF-I standard from the vial of Item C, into a tube with 350 µL of Assay Diluent C to prepare a 30 ng/mL standard solution. Pipette 300 µL of Assay Diluent C into each tube. Use the 30 ng/mL standard solution to produce a dilution series (see Figure 1). Mix each tube thoroughly before the next transfer. Assay Diluent C serves as the zero standard (0 ng/mL).

Figure 1.
Dilution Series for Standards



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C+ 400 µl	350 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl		300 µl
Conc.	100 ng/ml	30 ng/ml	12 ng/ml	4.8 ng/ml	1.92 ng/ml	0.768 ng/ml	0.307 ng/ml	0.123 ng/ml		0 ng/ml

5. 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.

6. 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 (Item L) and used in Procedure, step 5.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 120-fold with Assay Diluent C.

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 100 µL of HRP-Streptavidin concentrate into a tube with 12 mL of Assay Diluent C to prepare a final 120-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Storage/Stability
Store the kit at –20 °C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles.

The reconstituted standard should be stored at –20 °C or –70 °C (–70 °C is recommended). Opened microplate strips or reagents may be store for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Procedure

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. Label removable 8 well strips as appropriate for the experiment.

3. Add 100 µL of each standard (see Preparation Instructions, step 3) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4 °C with gentle shaking.

4. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with 1x Wash Buffer (300 µL) using a multichannel 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.

5. Add 100 µL of 1x prepared biotinylated antibody (Preparation Instructions, step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µL of prepared Streptavidin solution (see Preparation Instructions, step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

10. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

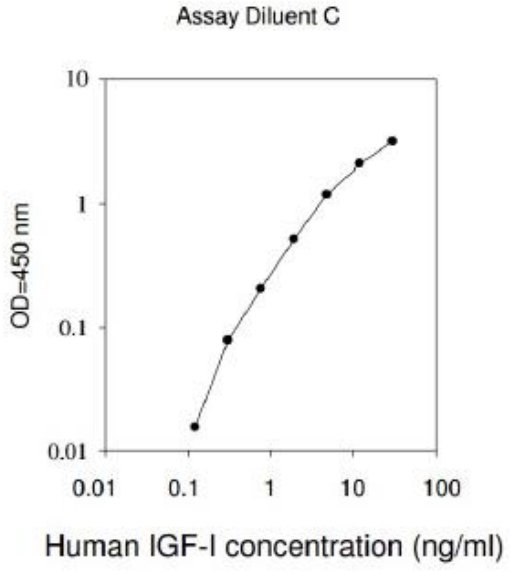
Results

Calculations

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.

Typical Data

Standard curve(s) is for demonstration only. A standard curve must be run with each assay.



Product Profile

Sensitivity: The minimum detectable dose of human IGF-I was determined to be 100 pg/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Reproducibility:

Intra-Assay: CV <10%

Inter-Assay: CV <12%

Spiking & Recovery:

Recovery was determined by spiking various levels of Human IGF-1 into the sample types listed below. Mean recoveries are as follows:

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

Linearity:

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

Specificity

This ELISA antibody pair only can detect a free form of human IGF-I (not a complex such as binding with IGFBPs).

This IGF-1 ELISA kit shows no cross-reactivity with any of the cytokines tested: 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, I-309, IP-10, G-CSF, GM-CSF, IFN-γ, Leptin (OB), MCP-1, MCP-3, MDC, MIP-1α, MIP-1β, MIP-1δ, PARC, RANTES, SCF, TARC, TGF-β, TIMP-1, TIMP-2, TNF-α, TNF-β, TPO, and VEGF.

Appendix

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 3 may be done over night at 4°C with gentle shaking Note: may increase overall signals including background.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Add stop solution to each well before reading plate.

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