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Human IGF-I ELISA Kit

Catalog No: CKH152A
CKH152B

Size: 1 x 96 tests
5 x 96 tests

Specificity:	Human IGF-I, free IGF-I, not complexed with IGFBPs
Sensitivity:	100 pg/ml, minimum detectable dose
Range:	0.123 ng/ml to 100 ng/ml
Sample Type:	Cell supernatants, serum, plasma samples.

Introduction:

The Human IGF-I ELISA is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human IGF-I in serum, plasma, and cell culture supernatants. This assay employs an antibody specific for 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-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.

Reagents and materials supplied in the kit:

Items	Quantity	Storage/Stability After Preparation
CKH152-A Anti-Human IGF-I Microplate: 12 strips x 8 wells	96 wells	1 month at 2-8°C
CKH152-B Wash Buffer Concentrate (20x)	25 mL	1 month at 2-8°C
CKH152-C Recombinant Human IGF-I Standard 1 vial is enough to run each standard in duplicate.	2 vials	1 week at -80°C
CKH152-D Detection Antibody: Biotinylated Anti-Human IGF-I Each vial is enough to coat ½ microplate.	2 vials	5 days at 2-8°C
CKH152-E Streptavidin-HRP Concentrate (120x)	200 µl	Do not store and reuse.
CKH152-F Assay Diluent C: 2 bottles, 30 ml each	60 mL	n/a
CKH152-G TMB One-Step Substrate Reagent (3, 3', 5, 5'-tetramethylbenzidine in buffered solution)	12 mL	n/a
CKH152-H Stop Solution (0.2 M Sulfuric Acid)	8 mL	n/a

Storage of Kit Reagents:
The entire kit may be stored for 6 months from date of shipment at 2-8°C or 1 year at -20°C. For extended stability, store at -80°C. For opened kit and prepared reagents, see Table above.

Materials not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- 100 mL and 1 liter graduated cylinders
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer/software for data analysis
- Tubes to prepare standard or sample dilution.

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Preparation of Kit Reagents:

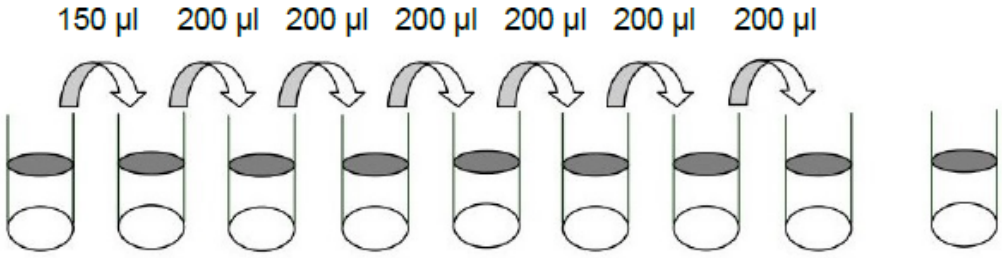
1.Bring all reagents and samples to room temperature (18-25°C) before use.

2. Assay Diluent C (**CKH152-F**) is ready-to-use.

3. Sample Dilution:
If samples need to be diluted, Assay Diluent C (**CKH152-F**) is used for dilution of serum, plasma, and cell culture supernatants. We suggested dilution of normal serum/plasma is 2-fold to 20-fold.
Note that levels of IGF-I may vary in specimens. Optimal dilution factors for each sample must be determined by the investigator.

4. **Preparation of Standard**:
Briefly centrifuge the vial of **CKH152-C** (Recombinant Human IGF-I Standard).
Add 400 µl Assay Diluent C to prepare a 100 ng/mL standard.
Dissolve the powder thoroughly by gentle mixing.
Add 150 µl standard from the vial of Item C, into a tube with 350 µl Assay Diluent C to prepare a 30 ng/mL stock standard solution. Add 300 µl Assay Diluent C into each tube. Use the stock standard solution to produce a dilution series (shown below in Figure 1).
Gently vortex to mix each tube thoroughly before the next transfer.
Assay Diluent serves as the zero standard (0 ng/mL).

Figure 1



		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. **Wash Buffer**:
If the Wash Concentrate (**CKH152-B**) contains visible crystals, warm to RT and mix gently until dissolved.
Dilute 20 mL of Wash Buffer Concentrate into distilled water to yield 400 mL of 1x Wash Buffer.

6. **Detection Antibody:**
Briefly centrifuge Detection Antibody vial (**CKH152-F**) before use.
Add 100 µl of Assay Diluent C into the vial to prepare a detection antibody concentrate.
Mix gently (the concentrate can be stored at 2-8°C for 5 days).
The detection antibody concentrate should be diluted 80-fold with Assay Diluent C and used in Step 5 of the Assay Procedure.

7. **Streptavidin-HRP:**
Briefly spin Streptavidin-HRP Concentrate vial (**CKH152-G**) and mix gently before use.
Streptavidin-HRP concentrate should be diluted 120-fold with Assay Diluent C, 100 µl HRP-Streptavidin + 12 ml Assay Diluent C. Mix well, use within same day.

ASSAY PROCEDURE:

Be sure to read '**Preparation of Kit Reagents**' before carrying out the assay.

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 (see **Reagent Preparation step 3**) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 2-8°C with gentle shaking.

3. Discard the solution and wash 4 times with 1x Wash Solution (300 µl each). Wash by filling each well using a multi-channel Pipette or auto-washer. 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 antibody (see **Reagent Preparation step 6**) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution and repeat the wash as in step 3.

6. Add 100 µl of prepared Streptavidin solution (see **Preparation of Kit Reagents step 7**) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution and repeat the wash as in step 3.

8. Add 100 µl of TMB One-Step Substrate Reagent (**CKH152-H**) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

9. Add 50 µl of Stop Solution (**CKH152-I**) to each well. Read at 450 nm immediately.

Assay Procedure Summary:

1. Prepare all reagents, samples and standards as instructed.

2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at RT.

3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at RT.

4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at RT.

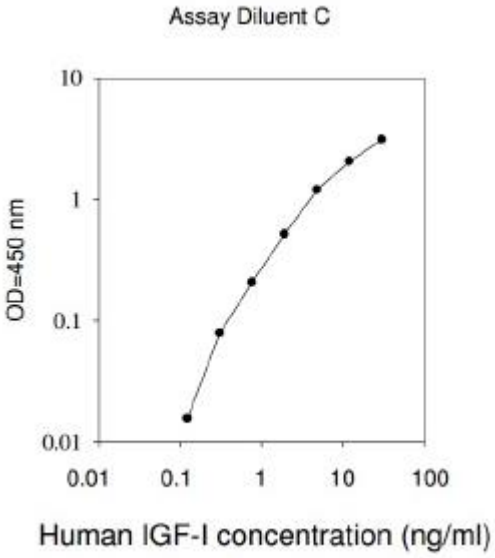
5. Add 100 µl TMB to each well. Incubate 30 minutes at RT.

6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

Calculation of Results:

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 is for demonstration **ONLY**. A standard curve **MUST** be run with each assay.



Performance and Characteristics:
Sensitivity:
The minimum detectable dose of Human IFG-I was determined to be 100 pg/mL.

Recovery:
Recovery was determined by spiking various levels of Human Angiogenin into Human serum, plasma and cell culture media. 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

Reproducibility:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Specificity:

This ELISA antibody pair detects the free form of IGF-I, not complexed with IGFBPs. The kit shows no cross-reactivity with any of the human cytokines tested: BDNF, BLC, ENA-78, FGF-4, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN gamma, Leptin (OB), MCP-1, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, PDGF, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.

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Troubleshooting Guide:

Problem	Cause	Solution
1. Standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times	1. Ensure sufficient incubation time; change Step 2 to overnight.
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation.
3. Large CV	1. Inaccurate pipetting	1. Check pipettes.
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer.
5. Low sensitivity	1. Improper storage	1. Store your standard at <-20°C after reconstitution, others at 2-4°C. Keep substrate solution protected from light.
	2. Stop solution	2. Stop solution should be added to each well before measure.

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