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Human Insulin-like Growth Factor Binding Protein 1 ELISA Kit

Catalog No: CKH148 **Size:** 1 x 96 tests

Introduction:

Insulin-like Growth Factor Binding Protein 1 (IGFBP1) is a member of the Insulin-like Growth Factor Binding Protein (IGFBP) family containing an IGFBP domain and a Thyroglobulin type-I domain. IGFBPs are found in various body fluids such as blood serum, amniotic fluid, and liquor. They are synthesized in the liver and are produced also by various tumor cell lines and cell types. IGFBP1 binds both Insulin-like Growth Factors (IGFs) 1 and 2 (IGF1 and IGF2) and circulates in the plasma. Binding of IGFBP1 prolongs the half-life of the IGFs and alters their interaction with cell surface receptors. IGFBP1 is found predominantly in the placenta and the amniotic fluid. It has been shown also to be an inhibitor of IGF mitogenic activities for human breast cancer cells. The effect of IGFBP1 depends on its phosphorylation status; phosphorylated IGFBP1 inhibits IGF actions whereas the non-phosphorylated isoform is stimulatory. Predominant sites of IGFBP1 transcription in the human fetal kidney are those with most active differentiation.

The Human IGFBP1 ELISA is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human IGFBP1 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for IGFBP1 coated on a 96-well plate. Standards and samples are pipetted into the wells and IGFBP1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and Biotinylated Anti-Human IGFBP1 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 IGFBP1 bound.

Performance and Characteristics:

Sensitivity

The minimum detectable dose of IGFBP1 is typically less than 5 pg/mL.

Reproducibility

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

Recovery

Recovery was determined by spiking various levels of Human IGFBP1 into Human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	95.27	84-104
Plasma	92.49	82-102
Cell culture media	94.64	83-102

Linearity

Sample Type		Serum	Plasma	Cell culture media
1:2	Average % of Expected	93	92	94
	Range (%)	81-101	82-102	83-103
1:4	Average % of Expected	94	93	93
	Range (%)	83-102	84-103	82-102
1:8	Average % of Expected	93	95	94
	Range (%)	82-102	83-102	84-104

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Reagents and materials supplied in the kit:

Items	Quantity
A. Microplate coated with Anti-Human IGFBP1	96 wells
B. Wash Buffer Concentrate (20x)	25 mL
C. Recombinant Human IGFBP1 Standards	2 vials
D. Assay Diluent A: Standard/Sample-Serum/Plasma *	30 mL
E. Assay Diluent B (5x): Standard/Sample-Cell Culture Medium/Urine	15 mL
F. Detection Antibody: Anti-Human IGFBP1	2 vials
G. Streptavidin-HRP Concentrate (12,000x)	8 µl
H. TMB One-Step Substrate Reagent (TMB in buffered solution)	12 mL
I. Stop Solution (2 M Sulfuric Acid)	8 mL

** Contains 0.09% Sodium Azide as preservative. Precaution: Sodium Azide is a poisonous and hazardous substance which should be handled by trained staff only.*

Storage of Kit Reagents:
Stable for up to 6 months from date of shipment at 2-4°C. Store reconstituted standard (recombinant protein) at -80°C. Opened Microplate Wells and reagents are stable for 1 month at 2-4°C. Return unused wells to the pouch containing desiccant pack and reseal along the entire edge.

Materials/reagents required but 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

Preparation of Kit Reagents:
Bring all reagents and samples to room temperature (18-25°C) before use.

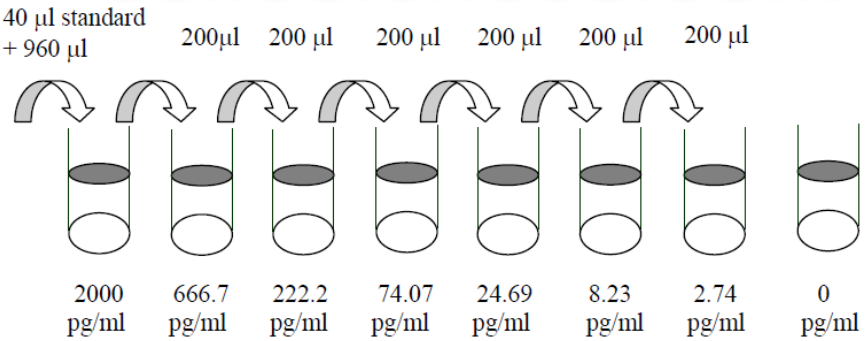
Sample Dilution
If your samples need to be diluted, use Assay Diluent A (Item D) for dilution of Serum/Plasma and Assay Diluent B (Item E) for Culture supernatants/Urine.

Assay Diluent
Dilute 5-fold with deionized or distilled water.

Wash Buffer Concentrate
- If the Wash Concentrate (Item 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.

IGFBP1 Standard
- Briefly spin the vial of Item C (Recombinant Human IGFBP1 Standard).
- Add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) to prepare a 50 ng/mL standard.
- Dissolve the powder thoroughly by a gentle mix.
- Add 40 µl standard from the vial of Item C, into a tube with 960 µl Assay Diluent A or 1x Assay Diluent B to prepare a 2000 pg/mL stock standard solution.
- Add 400 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below in Figure 1).
- Mix each tube thoroughly before the next transfer. Gently vortex to mix.
- Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL).

Figure 1



Detection Antibody

- Briefly spin Detection Antibody vial (Item F) before use.
- Add 100 µl of 1x Assay Diluent into the vial to prepare a detection antibody concentrate.
- Mix gently (the concentrate can be stored at 2-4°C for 5 days).
- The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent and used in Step 4 of the **ELISA Method**.

Streptavidin-HRP Concentrate

- Briefly spin Streptavidin-HRP Concentrate vial (Item G) and mix gently before use.
- Streptavidin-HRP concentrate should be diluted 12,000-fold with 1x Assay Diluent.

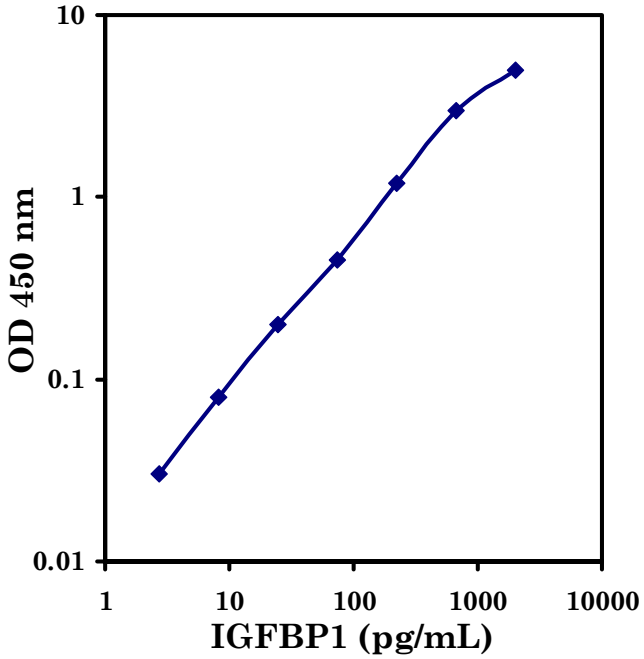
For example: Briefly spin the vial (Item G) and mix gently. Add 2 µl of Streptavidin-HRP concentrate into a tube with 198.0 µl 1x Assay Diluent B to prepare a 100-fold diluted Streptavidin-HRP solution (do not store the diluted solution for next day use). Mix thoroughly and then add 100 µl of prepared 100-fold diluted solution into a tube with 12 mL 1x Assay Diluent B to prepare a final 12,000 fold diluted Streptavidin-HRP solution.

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 or overnight at 2-4°C.
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.

Typical Data:

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



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ELISA Method:
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 **Preparation of Kit Reagents: IGFBP1 Standard**) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 2-4°C.

3. Discard the solution and wash 4 times with 1x Wash Solution (300 µl each).

4. Add 100 µl of 1x prepared biotinylated antibody (see **Preparation of Kit Reagents: Detection Antibody**) to each well. Incubate for 1 hour at room temperature.

5. Discard the solution and wash 4 times with 1x Wash Solution (300 µl each).

6. Add 100 µl of prepared Streptavidin solution (see **Preparation of Kit Reagents: Streptavidin-HRP Concentrate**) to each well. Incubate for 45 minutes at room temperature.

7. Discard the solution and wash 5 times with 1x Wash Solution (300 µl each).

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

9. Add 50 µl of Stop Solution (Item I) 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.

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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