IGFBP4 (Human) ELISA Kit

Catalog Number KA1873

96 assays

Version: 02

Intended for research use only

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Introduction

Background

IGF-BPs (Insulin-like growth factor binding proteins) 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. IGFBP-4 is the predominant IGF binding proteins expressed by human osteoblast-like cells. It is identical with a protein known as Colon cancer cell growth inhibitor. IGFBP-4 has been overexpressed in the malignant M12 prostate epithelial cell line to determine the effects on tumor formation and apoptosis. Overexpressing cells show reduced proliferation in response to IGF and inhibited colony growth.

Principle of the Assay

The IGFBP4 (Human) ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IGFBP-4 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human IGFBP-4 coated on a 96-well plate. Standards and samples are pipetted into the wells and IGFBP-4 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IGFBP-4 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 IGFBP-4 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

General Information

Materials Supplied

Component	Amount
IGFBP-4 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human IGFBP-4.	96 (8x12) wells
Wash Buffer Concentrate (20x) (Item B): 20x concentrated solution	25 ml
Standards (Item C): Recombinant human IGFBP-4.	2 vials
Assay Diluent A (Item D): Animal serum with 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.	30 ml
Assay Diluent B (Item E): 5x concentrated buffer. For Standard/Sample (cell culture medium/urine) diluent.	15 ml
Detection Antibody IGFBP-4 (Item F): Biotinylated anti-human IGFBP-4 (each vial is enough to assay half microplate).	2 vials
HRP-Streptavidin Concentrate (Item G): 600x concentrated HRP-conjugated streptavidin.	200 µl
TMB One-Step Substrate Reagent (Item H): 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.	12 ml
Stop Solution (Item I): 2 M sulfuric acid.	8 ml

Storage Instruction

May be stored for up to 6 months at 2 to 8℃ from the date of shipment. Standard (recombinant protein) should be stored at -20℃ or -80℃ (recommended at -80℃) after reconstitution. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8℃. 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℃. Avoid repeated freeze-thaw cycles.*

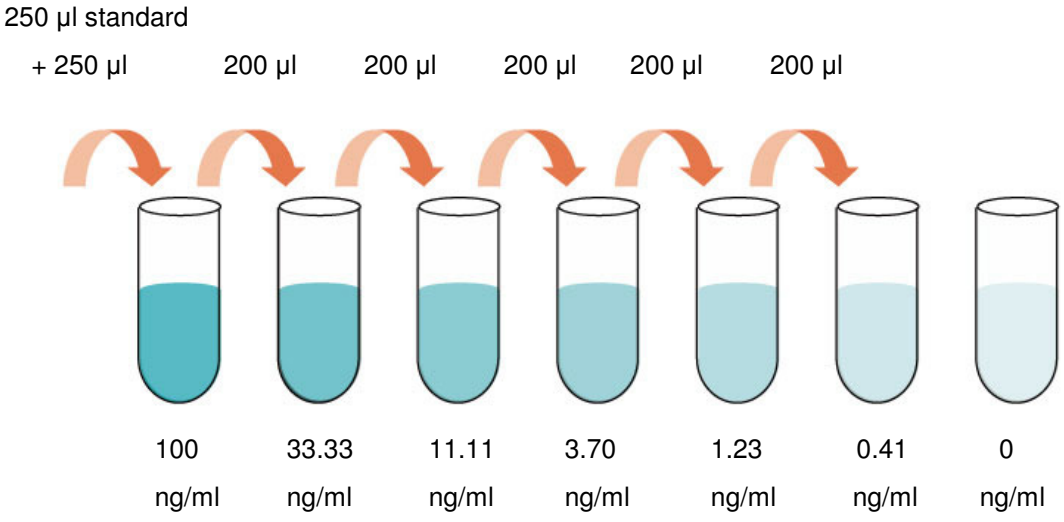
Materials Required but Not Supplied

- ✓ 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 and software for ELISA data analysis.
- ✓ Tubes to prepare standard or sample dilutions.

Assay Protocol

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25℃) before use.
2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernatants and urine.
   Suggested dilution for normal serum/plasma: 2-10 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.
3. Assay Diluent B should be diluted 5-fold with deionized or distilled water.
4. Preparation of standard: Briefly spin the vial of Item C and then add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) into Item C vial to prepare a 0.2 µg/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 250 µl IGFBP-4 standard from the vial of Item C, into a tube with 250 µl Assay Diluent A or 1x Assay Diluent B to prepare a 100.0 ng/ml stock standard solution. Pipette 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). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (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 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4℃ for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay

Diluent B and used in step 4 of Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 600-fold with 1x Assay Diluent B.
   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 25 µl of HRP-Streptavidin concentrate into a tube with 15 ml 1x Assay Diluent B to prepare a final 600-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25℃) 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 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4℃ 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 antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
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 with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

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 room temperature or over night at 4℃.
3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

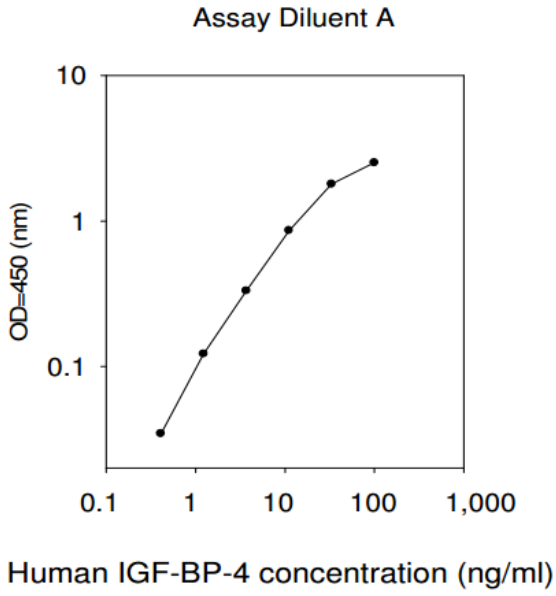
Data Analysis

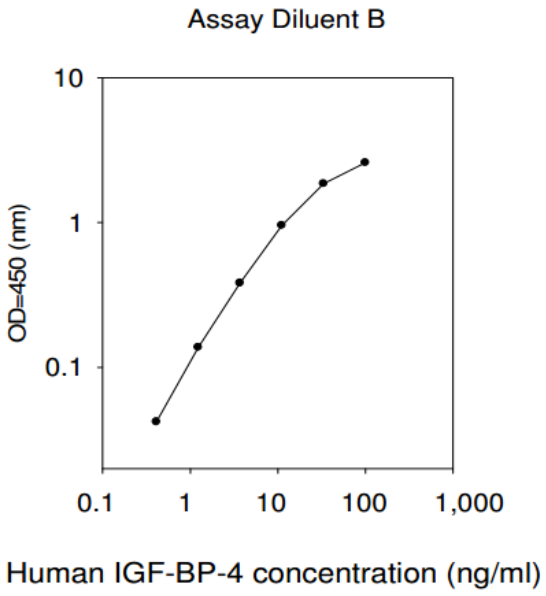
Calculation of Results

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

These standard curves are for demonstration only. A standard curve must be run with each assay.





Performance Characteristics

- Sensitivity

  The minimum detectable dose of IGFBP-4 is typically less than 0.25 ng/ml.

- Recovery

  Recovery was determined by spiking various levels of human IGFBP-4 into human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	93.98	83-105
Plasma	94.69	94-104
Cell culture media	97.38	86-106

- Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	93	94	95
	Range (%)	85-103	84-105	85-106
1:4	Average % of Expected	94	94	96
	Range (%)	86-105	85-105	87-107

- Ducibility

  Intra-Assay: CV<10%

  Inter-Assay: CV<12%

- Specificity

  Cross Reactivity: This ELISA kit 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, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, 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, MCP-1, MCP-2, MCP-3, MDC, MIP-1α, MIP-1 β, MIP-1δ, PARC, PDGF, RANTES, SCF, TARC, TGF-β, TIMP-1, TIMP-2, TNF-α, TNF-β, TPO, VEGF).

Resources

Troubleshooting

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

References

1. Angervo M et al. Epidermal growth factor enhances insulin-like growth factor binding protein-1 synthesis in human hepatoma cells. Biochemical and Biophysical Research Communications 189: 1177-83 (1992).
2. Damon SE et al. Overexpression of an inhibitory insulin-like growth factor binding protein (IGFBP), IGFBP-4, delays onset of prostate tumor formation. Endocrinology 139(8): 3456-3464 (1998).
3. Culouscou JM and Shoyab M. Purification of a colon cancer cell growth inhibitor and its identification as an insulin-like growth factor binding protein. Cancer Research 51: 2813-9 (1991).
4. Cohen P et al. Clinical aspects of insulin-like growth factor binding proteins. Acta Endocrinol. 124, Suppl. 2: 74-85 (1991).

Plate Layout

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