Datasheet for ABIN624999

IGFBP3 ELISA Kit

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Overview

Quantity:	96 tests
Target:	IGFBP3
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	80-18000 pg/mL
Minimum Detection Limit:	80 pg/mL
Application:	ELISA

Product Details

Purpose:	Human IGFBP-3 ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with any of the cytokines tested: Human ANG, CD23, Eotaxin, GCSF, GMCSF, GRO-alpha, GRO-beta, GRO-gamma, I-309, IFN-gamma, IGFBP-1, IGFBP-2, IGFBP-4, IL-1 alpha, IL-1 beta, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-15, IL-16, IP-10, MCP-1, MCP-2, MCP-3, MCP-4, MCSF, MIG, MIP-1 alpha, MIP-1 beta, NAP-2, PDGF, PF-4, PARC, SCF, SDF-1 alpha, TIMP-1, TIMP-2, TNFbeta, TGFbeta1, TGFbeta2, TGFbeta3, VEGF.
Sensitivity:	< 80 pg/mL

Product Details

Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments • Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard • Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	IGFBP3
Alternative Name:	IGFBP-3 (IGFBP3 Products)
Background:	The Human IGFBP-3 ELISA (Enzyme-Linked mmunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IGFBP-3 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human IGFBP-3 coated on a 96-well plate. Standards and samples are pipetted into the wells and IGFBP-3 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IGFBP-3 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-3 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	3486

Target Details

UniProt:	P17936
Pathways:	Myometrial Relaxation and Contraction, Regulation of Muscle Cell Differentiation, Skeletal Muscle Fiber Development, Regulation of Carbohydrate Metabolic Process, Autophagy, Smooth Muscle Cell Migration, Growth Factor Binding

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples20 - 200 fold
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared biotin antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared Streptavidin solution to each well. 7. Incubate 45 min at RT. 8. Add 100 µL of TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL of Stop Solution to each well. 11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18-25 °C) before use. 2. Sample dilution: If your samples need to be diluted, Standard /Sample Diluent (Item D) should be used for dilution of serum/ plasma/culture supernatants/samples. Suggested dilution for normal serum/plasma: 20-200 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 before use. 4. Preparation of standard: Briefly spin the vial of Item C. Add 400 µL Standard /Sample Diluent (Item D) to prepare a 0.1 ug/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 120 µL IGFBP-3 standard from the vial of Item C, into a tube with 546.7 µL Standard /Sample Diluent to prepare a 18000 pg/mL stock standard solution. Pipette 400 µL Standard /Sample Diluent into each tube. Use the stock standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. Standard /Sample Diluent serves as the zero standard (0 pg/mL). 200 µL 120 µL standard +546.7 µL 200myl 200 µL 200 µL 200 µL 18000 6000 2000 666.7 222.2 74.07 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/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 (Assay Diluent B should be diluted 5-fold with deionized or distilled water before use) 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 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure. 7. 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 320-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 50 µL of HRP-Streptavidin concentrate into a tube with 16 ml 1x Assay Diluent B to prepare a final 320 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 °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 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night 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 myl) 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 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 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.
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

Application Details

	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. Standard/Sample Diluent Human IGF-BP-3 concentration (pg/mL) O D =4 50 n m 0.001 0.01 0.1 1 10 10 100 1,000 10,000 100,000 Sensitivity: The minimum detectable dose of IGFBP-3 is typically less than 80 pg/mL. Recovery: Recovery was determined by spiking various levels of human IGFBP-3 into human serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 97.46 85-105 Plasma 96.69 84-106 Cell culture media 97.46 86-107 Linearity: Sample Type Serum Plasma Cell culture media 1:2 Average % of Expected 95 94 94 Range (%) 84-103 85-103 85-106 1:4 Average % of Expected 96 95 95 Range (%) 85-104 86-105 87-105 Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Publications

Product cited in:	Aucouturier, Thivel, Isacco, Fellmann, Chardigny, Duclos, Duché: "Combined food intake and exercise unmask different hormonal responses in lean and obese children." in: **Applied physiology, nutrition, and metabolism = Physiologie appliquée, nutrition et métabolisme**, Vol. 38, Issue 6, pp. 638-43, (2013) (PubMed). Schlatzer, Sugalski, Chen, Barnholtz-Sloan, Davitkov, Hazlett, Funderburg, Rodriguez, Lederman, Sieg, Chance, Anthony: "Plasma proteome analysis reveals overlapping, yet distinct mechanisms of immune activation in chronic HCV and HIV infections." in: **Journal of acquired**

immune deficiency syndromes (1999), Vol. 63, Issue 5, pp. 563-71, (2013) (PubMed).

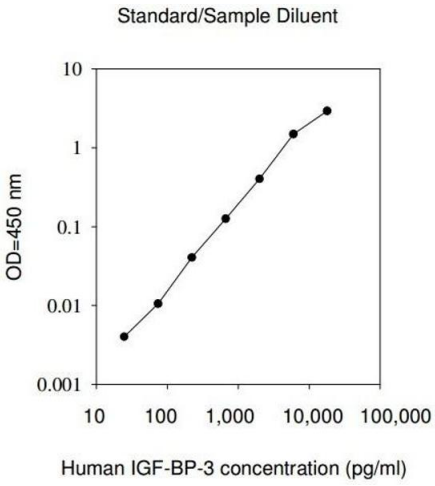
Brosseau, Pirianov, Colston: "Role of insulin-like growth factor binding protein-3 in 1, 25-dihydroxyvitamin-d 3 -induced breast cancer cell apoptosis." in: **International journal of cell biology**, Vol. 2013, pp. 960378, (2013) (PubMed).

Dokmanovic, Shen, Bonacci, Hirsch, Wu: "Trastuzumab regulates IGFBP-2 and IGFBP-3 to mediate growth inhibition: implications for the development of predictive biomarkers for trastuzumab resistance." in: **Molecular cancer therapeutics**, Vol. 10, Issue 6, pp. 917-28, (2011) (PubMed).

Kim, Hur, Min, Lee, Chung, Kim: "Inflammatory and tumor stimulating responses after laparoscopic sigmoidectomy." in: **Yonsei medical journal**, Vol. 52, Issue 4, pp. 635-42, (2011) (PubMed).

There are more publications referencing this product on: Product page

Images



ELISA

Image 1.