



Product Information Sheet

Human IGFBP-3 ELISA Kit

Catalog No. EK0386

Size 96T

Range 156.2pg/ml-10,000pg/ml

Sensitivity < 10 pg/ml

Specificity

No detectable cross-reactivity with any other cytokine.

Storage

Store at 4°C for frequent use, at -20°C for infrequent use.

Avoid multiple freeze-thaw cycles
(Shipped with wet ice.)

Expiration

Four months at 4°C and eight months at -20°C.

Application

For quantitative detection of human IGFBP-3 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle

Antagene's human IGFBP-3 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human IGFBP-3 specific-specific polyclonal antibodies were precoated onto 96-well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human IGFBP-3 amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant human IGFBP-3 standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- human IGFBP-3 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human IGFBP-3 antibody: 130μl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC): 130μl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

Material Required But Not Provided

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).

Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450μl of purified acetic acid or 700μl of concentrated hydrochloric acid to 1000ml H₂O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M PBS: Add 8.5g sodium chloride, 1.4g Na₂HPO₄ and 0.2g NaH₂PO₄ to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

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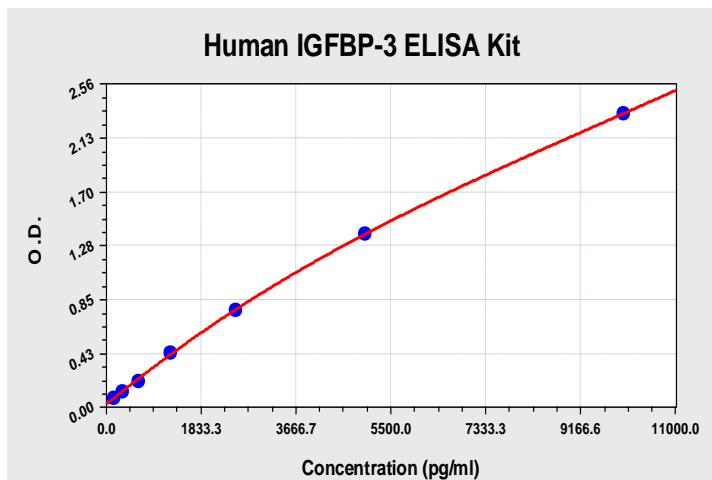
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Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

Human IGFBP-3 ELISA Kit-1X96 Well Plate Image



Background

Insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) is a major determinant of circulating levels of the IGFs and is clinically useful for the evaluation of GH deficiency and for predicting the response to GH treatment. The circulating level of IGFBP-3 is inversely related to the risk of several common cancers, and that antiproliferative agents such as antiestrogens and retinoids act in part by up-regulating IGFBP-3 gene (IGFBP3) expression.¹ Insulin-like growth factor-binding protein (IGFBP)-3, well characterized as the carrier of insulin-like growth factor (IGF), has been reported to have intrinsic bioactivity that is independent of IGF binding. IGFBP-3 has an IGF-independent, antiproliferative effect in undifferentiated and early differentiated but not in terminally differentiated chondrocytes.² IGFBP-3 possesses both growth-inhibitory and potentiating effects on cells that are independent of IGF action and are mediated through specific IGFBP-3 binding proteins/receptors locate at the cell membrane, cytosol, or nuclear compartments and in the extracellular matrix.³ IGFBP3 is also located on chromosome 7.⁴ The standard product used in this kit is recombinant human IGFBP-3, consisting of 265 amino acids with the molecular mass of 29kDa. As a result of glycosylation, the molecular mass is 41kDa.

Reference

1. Deal, C.; Ma, J.; Wilkin, F.; Paquette, J.; Rozen, F.; Ge, B.; Hudson, T.; Stampfer, M.; Pollak, M. Novel promoter polymorphism in insulin-like growth factor-binding protein-3: correlation with serum levels and interaction with known regulators. *J. Clin. Endocr. Metab.* 86: 1274-1280, 2001.
2. Spagnoli, A.; Torello, M.; Nagalla, S. R.; Horton, W. A.; Pattee, P.; Hwa, V.; Chiarelli, F.; Roberts, C. T., Jr.; Rosenfeld, R. G. Identification of STAT-1 as a molecular target of IGFBP-3 in the process of chondrogenesis. *J. Biol. Chem.* 277: 18860-18867, 2002.
3. Weinzimer, S. A.; Gibson, T. B.; Collett-Solberg, P. F.; Khare, A.; Liu, B.; Cohen, P. Transferrin is an insulin-like growth factor-binding protein-3 binding protein. *J. Clin. Endocr. Metab.* 86: 1806-1813, 2001.
4. Ehrenborg, E.; Larsson, C.; Stern, I.; Janson, M.; Powell, D. R.; Luthman, H. Contiguous localization of the genes encoding human insulin-like growth factor-binding proteins 1 (IGBP1) and 3 (IGBP3) on chromosome 7. *Genomics* 12: 497-502, 1992.

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