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Biotinylated Human IGFBP-4

Catalog Number: BP430-G2.5

Size: 2.5 µl

Storage: -20°C

PRODUCT SPECIFICATION SHEET

Product Specifications Biotinylated Human IGFBP-4

- Biotinylated human IGFBP-4 has been prepared from receptor grade recombinant human IGFBP-4. The product was purified using chromatographic techniques.
- **Appearance:** Lyophilized with no additives.
- **Reconstitution:** Reconstitute 2.5 µg aliquot with 0.1 ml double distilled water or buffer of your choice.
- **Storage:** The product is shipped at room temperature, for long term storage store at -20 °C. Reconstituted samples can be stored for at least one week at 2-8 °C.

Reference on the use of biotinylated IGFBP-4:

1.) Khawaja, X. Z.: Development of a scintillation proximity assay for human insulin-like growth factor-binding protein 4 compatible with inhibitor high-throughput screening. Analytical Biochemistry, Volume 366, Issue 1, 1 July 2007, Pages 80-86

Link to Abstract:

http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6W9V-4N919PB-2&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_version=1&_urlVersion=0&_userid=10&md5=a69f5f8529949e716d860ce3a5e5fd76

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Application Notes: Applications of Biotinylated (Biotin-labeled) IGFBP's.

Biotinylated IGF's and analogs have found a wide range of applications in in-vitro and in-vivo and are a safe and stable alternative to ¹²⁵I-labeled IGF's. As ¹²⁵I-labeled IGFBP's have also been used for methods as e.g. proteolysis assays, binding studies and in-vivo studies, there should be some potential for biotinylated IGFBP's, too. A literature search on the use of biotinylated IGFBP's resulted in a small number of reports, which are limited to IGFBP-1(1-3), IGFBP-2 (4,5), IGFBP-3 (6-14, 16) and IGFBP-4 (15). Until today we found no scientific papers on the use of biotinylated IGFBP-5 and IGFBP-6.

Though there is small number of papers, the authors have used the biotinylated IGFBP's in a broad range of techniques to study IGF-dependent and IGF-independent actions of IGFBP's. Biotinylated IGFBP's have been used in immunoassays (1, 6, 11, 15), proteolysis studies (2,3), ligand blot (4,10, 12, 16), cross-linking studies (9, 12) and binding studies (1, 4, 5, 7, 8, 13). Biotinylated IGFBP-3 has been used to demonstrate the nuclear appearance of IGFBP-3 in-vivo (14).

Studies with Biotinylated IGFBP's from IBT Systems/Eagle Biosciences:

To prove that biotinylated IGFBP's from IBT Systems/Eagle Biosciences are useful tools to demonstrate IGF dependent or independent actions of IGFBP's we used IGFBP-3 as an example. IGFBP-3 was biotinylated using the same technology as for our biotinylated IGF's.

- Detection of Biotinylated IGFBP-3 on Western Blots with Streptavidin-Peroxidase and Monoclonal Antibodies
 - The sensitivity in western blotting was determined by separation by SDS-PAGE, capillary blot to a nitrocellulose sheet (as described in application note IGF005). The blot was blocked in TBS-Tween, 5 % non fat dry milk for 30 minutes and incubated with Streptavidin-Peroxidase conjugate (dilution 1:2500) for 1 h. The blot was washed three time for five minutes in blocking buffer, followed by 1 wash with TBS and first incubated with the colorimetric TMB substrate (fig. 1) or with a chemiluminescent substrate (fig 2). A broad band was found at around 43 - 45 kDa. The sensitivity was equal or less than 0.5 ng. With the colorimetric substrate the 0.5 ng band appeared after ten minutes. The exposition time with the chemiluminescent substrate was five seconds. In the chemiluminescent blot dimers were visible, as we have observed them with non-biotinylated IGFBP-3 in our experiments with non-radioactive ligand blots.
 - The detection of biotinylated IGFBP-3 by a monoclonal antibody is described in detail in Application Note IGF018.
- Detection of Biotinylated IGFBP-3 in ELISA format. Biotinylated IGFBP-3 is detected in ELISA Format.
- Use of Biotinylated IGFBP-3 as a Substrate for Proteolysis
 - Biotinylated IGFBP-3 can be used as a substrate for IGFBP-3 proteases in ELISA format. For details see Application Note IGF016. Proteolysis with Plasmin is



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slower compared to non-biotinylated IGFBP-3. However the fragments obtained are equivalent in molecular weight. For details see Application Note IGF018.

- Western-ligand Blotting with Biotinylated IGFBP-5: interaction with Proteins from Tumor Cell Lines.
 - As we found no reference on the use of biotinylated IGFBP-5, we have used IGFBP-5 for ligand blotting experiments with extracts from cancer cell lines. A blot with total cell extracts from Jurkat (Acute T cell Leukemia), K562 (Chronic myelogenous Leukemia), MCF7 (Breast Cancer), Raji (B lymphoma) cells and normal human placenta as a control was incubated overnight with 100 ng/ml biotinylated IGFBP-5 in 5 % non-fat dry milk in TTBS, followed by incubation with Streptavidin Peroxidase (1:1000): Detection was done with a chemiluminescent substrate and exposition to a Polaroid film (as explained in detail in Application Note IGF005) and an X-ray film (following the instructions of the manufacturer of the substrate).
 - The blot showed a complex pattern of binding of biotinylated IGFBP-5 to proteins from the tumor cell lines, but not from the normal tissue. Though the blotting and detection procedure needs to be improved and a much more detailed analysis of the binding of biotinylated IGFBP-5 to proteins from tumor and normal cells is necessary, the example demonstrates, that biotinylated IGFBP-5 may be a valuable tool in western-ligand blotting and other techniques

References:

1.) Lowman et.al.: Mapping the Binding Epitopes of IGF-I and a phage-library derived peptide that inhibits IGFBP-1 binding to insulin-like growth factor. Peptides for the New Millennium. Proceedings of the 16th American Peptide Symposium June 26–July 1, 1999, Minneapolis, Minnesota, U.S.A. 10.1007/0-306-46881-6_245. Edts. Gregg B. Fields, James P. Tam and George Barany.

Link to Abstract:

Mapping the binding epitopes of IGF-1 and a phage-library derived peptide that inhibits IGFBP-1 binding to insulin-like growth factor

A preview can be seen at:

<http://books.google.com/books?id=JCl1c6Ps85kC&pg=PT691&lpg=PT691&ots=DAIgXTunl9&dq=%22Biotinylated+IGFBP-%22&ie=ISO-8859-1&output=html&sig=ACfU3U1VNQNNMAd1quTSuN4J7uctAcHewg>

2.) Wang et. al.: Specific cleavage of insulin-like growth factor-binding protein-1 by a novel protease activity. Cellular and Molecular Life Sciences (CMLS). Volume 63, Numbers 19-20 / Oktober 2006.

Link to Abstract: <http://cat.inist.fr/?aModele=afficheN&cpsidt=18241019>

3.) Wang, J.: Novel Insulin-like Growth Factor Binding Protein Proteases: Detection and Characterization. Thesis at the Karolinska Institutet 2006.



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Link to Thesis: <http://diss.kib.ki.se/2006/91-7140-942-4/thesis.pdf>

4.) Pereira et al.: Bimolecular Interaction of Insulin-Like Growth Factor (IGF) Binding Protein-2 with $\alpha\beta 3$ negatively Modulates IGF-I-Mediated Migration and Tumor Growth. *CANCER RESEARCH* 64, 977–984, February 1, 2004.

Link to article: <http://cancerres.aacrjournals.org/cgi/reprint/64/3/977.pdf>

5.) Ben et al.: DIFFERENTIATIVE EFFECTS OF IGF SYSTEM COMPONENTS ON BOVINE FOLLICULAR CELLS. *Biology of Reproduction*, Volume 64, Supplement 1. Society for the Study of Reproduction, 34th Annual Meeting, July 28 - August 1, 2001 University of Ottawa.

Link to abstract: <http://abstracts.co.allenpress.com/pweb/ssr2001/document/29588>

6.) Puvogel et al.: Effects of an Enhanced Vitamin A Intake During the Dry Period on Retinoids, Lactoferrin, IGF System, Mammary Gland Epithelial Cell Apoptosis, and Subsequent Lactation in Dairy Cows. *J. Dairy Sci.* 88:1785–1800. American Dairy Science Association, 2005.

Link to article: <http://jds.fass.org/cgi/reprint/88/5/1785>

7.) Leal et al.: Interactions of High Affinity Insulin-like Growth Factor-binding Proteins with the Type V Transforming Growth Factor- β Receptor in Mink Lung Epithelial Cells. *THE JOURNAL OF BIOLOGICAL CHEMISTRY* Vol. 274, No. 10, Issue of March 5, pp. 6711–6717, 1999.

Link to article: <http://www.jbc.org/cgi/reprint/274/10/6711>

8.) Suresh, M. and Murphy L.M.: Phosphorylation of Insulin-Like Growth Factor (IGF) Binding Protein-3 by Breast Cancer Cell Membranes Enhances IGF-I Binding. *Endocrinology* Vol. 144, No. 9 4042-4050.

Link to article: <http://endo.endojournals.org/cgi/content/full/144/9/4042#F2>

9.) Suresh, M. and Murphy L.M.: The effects of insulin-like growth factor binding protein-3 (IGFBP-3) on T47D breast cancer cells enriched for IGFBP-3 binding sites. *Mol Cell Biochem.* 2004 Dec;267(1-2):83-9.

Link to abstract: <http://www.ncbi.nlm.nih.gov/pubmed/15663189>

10.) Gui, Y and Murphy, L. M.: Insulin-Like Growth Factor (IGF)-Binding Protein-3 (IGFBP-3) Binds to Fibronectin (FN): Demonstration of IGF-I/IGFBP-3/FN Ternary Complexes in Human Plasma. *The Journal of Clinical Endocrinology & Metabolism* Vol. 86, No. 5 2104-2110.

Link to article: <http://jcem.endojournals.org/cgi/content/full/86/5/2104>

11.) Brand-Miller et al.: The glycemic index of foods influences postprandial insulin-like growth factor-binding protein responses in lean young subjects. *American Journal of Clinical Nutrition*, Vol. 82, No. 2, 350-354, August 2005

Link to article: <http://www.ajcn.org/cgi/content/full/82/2/350>



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12.) Mishra et al.: Insulin-Like Growth Factor Binding Protein-3 Interacts with Autocrine Motility Factor/Phosphoglucose Isomerase (AMF/PGI) and Inhibits the AMF/PGI Function. *CANCER RESEARCH* 64, 2516–2522, April 1, 2004

Link to article: <http://cancerres.aacrjournals.org/cgi/reprint/64/7/2516.pdf>

13.) Suresh, M. and Murphy L.M.: IDENTIFICATION OF TRANSGLUTAMINASE 2 AS AN INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-3 KINASE. *THE JOURNAL OF BIOLOGICAL CHEMISTRY* Vol. 279, No. 23, Issue of June 4, pp. 23863–23868, 2004

Link to article: <http://www.jbc.org/cgi/reprint/279/23/23863>

14.) Baumrucker et al.: The Insulin-like Growth Factor (IGF) System in Mammary Gland: Role of IGFBP-3 Binding Protein. In: *Ruminant Physiology: Digestion, Metabolism, Growth, and Reproduction*. By Pierre Cronjé, E. A. Boomker. Contributor Pierre Cronjé. Published by CABI Publishing, 2000, ISBN 0851994636, 9780851994635

A preview of the book is available at:

http://books.google.com/books?id=_cxUuEaITRsC&pg=PA343&lpg=PA343&dq=biotinylated+IGF-&source=web&ots=DW09t7fwLM&sig=W4uDm1hxfPevSdjixJwCkHkJmoc&hl=en&sa=X&oi=book_result&resnum=5&ct=result

15.) Khawaja, X. Z.: Development of a scintillation proximity assay for human insulin-like growth factor-binding protein 4 compatible with inhibitor high-throughput screening. *Analytical Biochemistry*, Volume 366, Issue 1, 1 July 2007, Pages 80-86

Link to Abstract:

http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6W9V-4N919PB-2&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_version=1&_urlVersion=0&_userid=10&md5=a69f5f8529949e716d860ce3a5e5fd76

16.) Campbell et al: Insulin-like Growth Factor-binding Protein-3 Binds Fibrinogen and Fibrin. *The Journal of Biological Chemistry* October 15, 1999, 274, 30215-30221.

Link to article: <http://www.jbc.org/content/274/42/30215.full>



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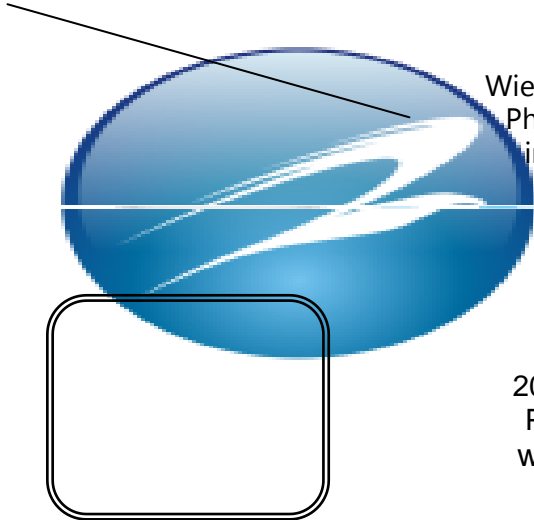
Catalog Number: BP430-G2.5

Size: 2.5 µl

Storage: -20°C

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