Biotinylated IGFBP-2 Recombinant

Catalog Number:
BG230-G2.5 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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Biotinylated Human Insulin-like Growth Factor 1 (IGF-1)

- **Description**:
  Biotinylated Human IGFBP-2 has been prepared from receptor grade recombinant human IGFBP-2.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.

Application Notes

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 125 I-lableld IGF´s. As 125 I-lableld 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 invivo (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 o 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 nonradioactive 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 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. o 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 IGFB005) and an X-ray film (following the instructions of the manufacturer of the substrate). o 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

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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:
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Storage: -20°C

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