Package Insert

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Biotinylated Rat IGFBP-4 Recombinant Protein

Catalog Number:
BP420-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 rat IGFBP-4 has been prepared from receptor grade recombinant rat IGFBP-4 (product code BP4CU020) using the IBT biotinylation technology. 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-vitro and in-vivo (see application note IGF004) and are a safe and stable alternative to 125I-labeled IGF´s. As 125I-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 (4,5,17), IGFBP-3 (6-14, 18) 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), 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) and as a substrate for transglutaminase (13).

Biotinylation Technology:

We have developed our own biotinylation technology for IGF´s that is different from the published methods. Our technology permits biotinylation without loss of binding capacity to IGFBP´s or IGF antibodies.

Studies with Biotinylated IGFBP's from IBT

To prove, that biotinylated IGFBP´s from IBT 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 (as described in notes below).

Stability
Though our biotinylated IGF´s are very stable in solution, we have shipped them in the past on dry ice to ensure maximum activity for our customers. This has increased shipping cost so we have decided to lyophilize our IGF´s. In this form we have kept them for three months at temperatures up to 35 °C without loss of activity. So they can be shipped now without dry ice at reduced freight cost.

For long term storage, we recommend to store the IGF's refrigerated after arrival. The reconstituted biotinylated IGF´s are stable for at least one year at a concentration of 20 µg/ml, if kept frozen at -20 °C, and at least for one month at 4 °C. We recommend to aliquot the reconstituted biotinylated IGF´s and to store them at a temperature of - 20 °C.

Potential Uses for Biotinylated IGF's

Western-Ligand Blotting
We have tested our biotinylated IGF´s and analogs in western-ligand blotting.
Chemiluminescent substrates as well as colorimetric ones have successfully been used. We also have developed our own buffer system, with buffers, that are stable for at least six months and give an excellent signal-to background ratio. Kits are available for human (bovine, sheep, donkey, pig, guinea pig, goat) IGFBP´s and mouse/rat IGFBP´s.

Western-Ligand blots with biotinylated IGF-I and analogs
Biotinylated IGF-I binds to all six IGFBP´s, but binds to IGFBP-6 very weakly. For all IGF-I analogs at least weak signals have been obtained with IGFBP-1 to -5, but no signals were observed with IGFBP-6.

ELISA
We have tested our biotinylated human IGF-I and IGF-II in a competitive ELISA format using our polyclonal antisera PAA1 and PAC1. Binding to the antibodies was not affected by biotinylation.

Immunoprecipitation/Chromatography of IGFBP's
We have used our biotinylated human IGF-I and IGF-II in immunoprecipitation experiments. Binding of biotinylated IGF´s to Streptavidin may also be used to prepare media for affinity chromatography in a column format.

Cross-linking Experiments
We have started to develop methods using our biotinylated IGF´s for cross-linking experiments. The method is not fully developed, e.g. the washing procedure needs to be improved and the extraction procedure for proteins needs improvement, too. Preliminary results are available.

Immunohistochemistry
We have started experiments with our biotinylated IGF´s for IHC with human cells fixed on slides. The results of histochemistry have not been examined systematically, but staining has been observed on the surface of the cells.

Flow Cytometry:
The use of Biotin-Des (1-3) IGF-I in flow cytometry has been reported by Xu et al. (1995), Immunology 85, 394-399.

References
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

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