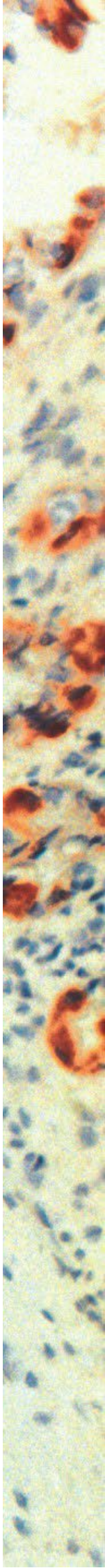




TECHNICAL BULLETIN #3

Biotinylated IGFs - Binding to IGFBPs & the Type 1 IGF Receptor



Biotinylated reagents are rapidly replacing traditional radioactive techniques for the sensitive, rapid and safe detection of an increasing number of biological molecules. To support this trend GroPep offers three high purity Biotinylated human IGFs.

Using NHS-biotin reagents which react with primary amines in the IGF molecule, labelled molecules with excellent binding to avidin-linked detection reagents were produced.

Biotinyl IGF-I

- The major species is di-biotinyl IGF-I. Also contains mono- and tri-biotinyl species.
- Prepared with a linker arm chosen to minimize steric hindrance during subsequent avidin binding.

Mono-biotinyl IGF-II

- Biotinyl group linked to the N-terminal alanine.

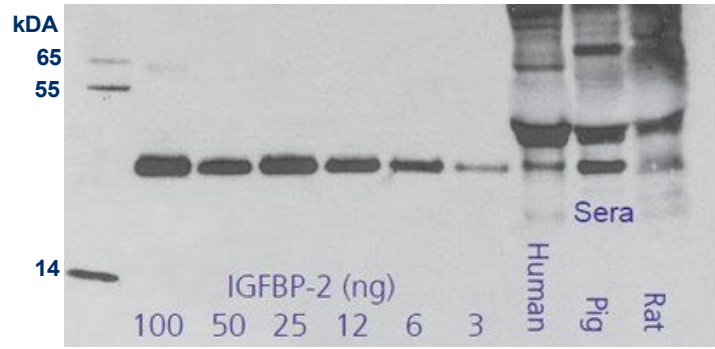
Di-biotinyl IGF-II

- Biotinyl groups attached to both the N-terminal alanine and lysine 65.
- Similar binding characteristics as mono-biotinyl IGF-II. (Confirmed by Western Ligand Blot).

Biotinyl IGFs are suitable for many applications. Two examples of their use are shown below. Other applications are envisaged, including as histochemical reagents.

1. Visualization of IGFBPs by Western Ligand Blot

With Biotinyl IGF-I:



With Mono-Biotinyl IGF-II:



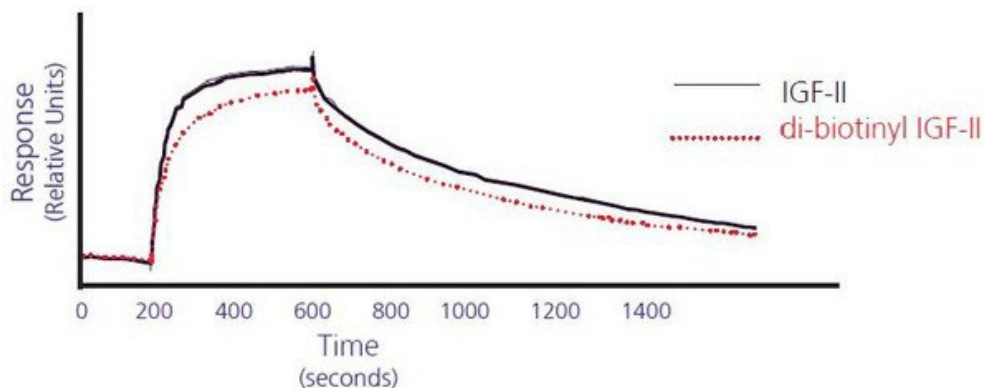
Gel: 10-20 % Tris-tricine SDS-PAGE, transferred to nitrocellulose
 Load: Serial dilution of bovine IGFBP-2. 2.5 µl each of human, pig and rat serum
 Probed with: 0.2 µg/ml biotinyl IGF-I or mono-biotinyl IGF-II
 Visualization: Streptavidin-horseradish peroxidase and chemiluminescent reagents
 Exposure time: 8 minutes

TECHNICAL BULLETIN #3

Biotinylated IGFs - Binding to IGFBPs & the Type 1 IGF Receptor

2. Type 1 IGF Receptor Binding

Soluble Type 1 IGF Receptor (Surinya et al., 1998) was immobilized on a CM5 chip and a 200 nM solution of either human IGF-II or di-biotinyl IGF-II passed over the receptor. This BIAcore analysis shows that both the native molecule and the biotinyl IGF-II exhibit similar association and dissociation rates, demonstrating equivalent binding to the IGF receptor.



Useful References

- Surinya, K. H., et al. (1998) Production and characterisation of a soluble high affinity IGF-I receptor. Proc. Aust. Soc. Biochem. Mol. Biol., 30, Pos. 132.
- Grulich-Henn, J., et al. (1998) Ligand blot analysis of insulin-like growth factor-binding proteins using biotinylated insulin-like growth factor-I. Hormone Res., 49, 1-7.
- Op De Beeck, L., et al. (1997) Detection of serum insulin-like growth factor binding proteins on western ligand blots by biotinylated IGF and enhanced chemiluminescence. J. Endocrinol., 154, R1-R5.
- Fowlkes, J. L. and Serra, D. (1996) A rapid, non-radioactive method for the detection of insulin-like growth factor binding proteins by Western ligand blotting. Endocrinol., 137, 5751-5754.

Biotinyl Human IGF-I

Code: AQU050 50 µg
Code: AQU100 100 µg
Code: AQU500 500 µg

Mono-Biotinyl Human IGF-II

Code: AMU010 10 µg
Code: AMU050 50 µg

Di-biotinyl Human IGF-II

Code: ANU010 10 µg



For orders and enquiries contact GroPep Bioreagents via:

Email: info@gropep.com

Phone: +61 8 7222 1051