

# Polysaccharides Conjugation using AnteoBind™ activated Luminex® Microspheres

AnteoTech Ltd.

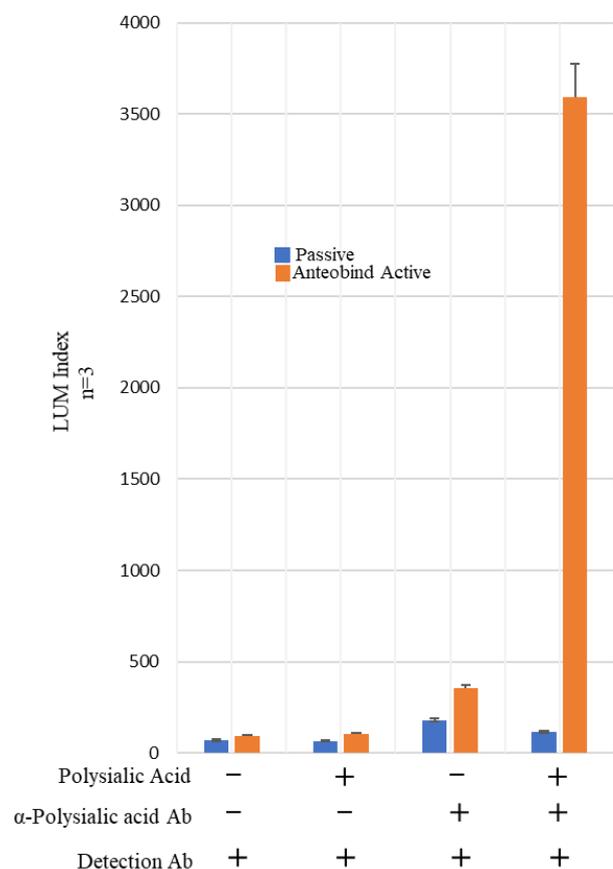
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The use of multiplex magnetic Luminex® microspheres for the simultaneous detection of several analytes has become more popular in immunoassays recently. Perhaps the most well accepted process of conjugating proteins or polysaccharides to the microspheres is using EDC chemistry. This process requires EDC and a primary amine for the conjugation of biomolecules to the microspheres. (Purohit, S. 2018; Rogier, E. 2019). In some cases, pH optimisation is required to improve the conjugation efficiency, which may adversely affect the activity of the biomolecules.

Polysaccharides have extensively been used in the vaccine industry. However, using conventional conjugation procedures can have certain challenges. AnteoBind™ technology is a rapid, single step process of activating the Luminex microspheres for the conjugation of biomolecules such as proteins or carbohydrate polymers. This process is designed to carry out the activation and conjugation of microspheres at room temperature and does not require any pH adjustment during the process. The conjugation of biomolecules to the microspheres does not require any chemical reactions and thereby maintaining the integrity of the biomolecules. Once the microspheres are activated, they can be stored and used over a long period of time without an observable drop in the binding efficiency.

AnteoBind has also been endorsed by Luminex corporation as an alternative method to overcome conjugation difficulties by binding proteins with metal chelation (A New Alternative For Coupling Your Problematic Proteins On Luminex® xMAP® Microspheres, Feb, 6, 2019). However, no observation has been reported so far about the efficacy of the AnteoBind in conjugating carbohydrate polymers to the Luminex microspheres.

We have recently investigated the conjugation of a carbohydrate polymer, Polysialic Acid (known as Colominic Acid, Sigma-Aldrich), with the Luminex microspheres activated with AnteoBind. We chose Polysialic Acid in the first instance because it is a commonly used poly-carbohydrate in the field of vaccine research. The binding of the Polysialic acid to the activated microspheres was detected by using antibodies against Polysialic Acid ( $\alpha$ -Polysialic acid Ab, Absolute Antibody, UK). Finally, to estimate the degree of binding, a secondary antibody coupled with a fluorophore (Detection Antibody) was used to determine the signal intensity using Luminex Bio-Plex 100 Array Reader. The same conjugation reaction was performed with the passive (without AnteoBind activation) microspheres. To determine the non-specific signals, the binding reactions were also performed in the presence (+) or absence (-) of either Polysialic Acid or  $\alpha$ -Polysialic acid antibody. Results, as shown in the figure, indicate that the activation of Luminex microspheres upregulated the binding of the microspheres with Polysialic Acid significantly. Several experiments were conducted with varying concentrations of Polysialic Acid to optimize the conjugation reaction.



This report demonstrates the binding of a poly-carbohydrate to Luminex microspheres using AnteoBind. Carbohydrates have been widely used in the last few decades

in vaccine development. They are considered as an attractive immune adjuvant through the activation of T helper cells. Currently, vaccines targeting capsular polysaccharides against bacteria and viruses are available but have been reported as being inefficient in protecting children and immunocompromised people (Lesinski, GB. et al.2001). AnteoBind technology, would accelerate the testing of carbohydrate or carbohydrate conjugated vaccine candidates based on the Luminex microsphere-based multiplex immunoassays, providing a fast, stable and a reproducible conjugation process.

#### **AnteoTech Offers Flexible Solutions to time consuming problems**

AnteoTech recognises some key bio-conjugation roadblocks in the Life Science industry. We believe resources and on time delivery of products are critical to the success of every business, hence why we are offering Bio-conjugation and assay development services to our industry partners using our proven alternative technology in the toolbox – AnteoBind.

Please contact us at [contact@anteotech.com](mailto:contact@anteotech.com) for further information on our services.

#### **References :**

A New Alternative For Coupling Your Problematic Proteins On Luminex® xMAP® Microspheres, Feb, 6, 2019.

[An Alternative For Coupling Your Problematic Proteins](#)

Lesinski GB, et al. *Curr Drug Targets Infect Disord.* 2001;1(3):325-334.

Purohit S, et al. *Nat Commun.* 2018;9(1):258.

Rogier E, et al. *Malar J.* 2019;18(1):402.