Biotinylated Autoantigens

Important in the development of novel high-throughput technologies in diagnostic platforms is to establish the highest possible sensitivity. Several factors influence sensitivity of an in-vitro diagnostic assay including the use of certain surface characteristics or reporter molecules to which the secondary antibody is conjugated. When the appropriate reporter molecule and surface material is chosen, even higher assay sensitivities can be obtained by biotin-conjugation of the antigen and subsequent incubation with a streptavidin-conjugated reporter molecule or coated surface (WHO 2006).

Binding of biotin to streptavidin / avidin is one of the strongest non-covalent interactions known in nature, which results from several factors. Those include the formation of multiple hydrogen bonds and van der Waals interactions between biotin and the protein, together with surface polypeptide loops that bury the biotin within the protein. Structural alterations at the biotin binding site produce quaternary changes in the streptavidin tetramer, which propagate formations in the twisted beta-sheets that link the tetramer subunits (Weber *et al.* 1989).

Some of the most recent IVD developments make use of streptavidin-coated surfaces to increase strength of attachment to the base. In multiplex bead-based systems biotinylated antigens are coupled on streptavidin beads, customarily magnetic. This principle of surface extension allows measurement of a large variety of antigens in one step. Furthermore, there is no need for large sample amounts. Performance of those assays is more efficient and less time-consuming, compared to standard ELISA or Western Blot / immunodot technologies.

For validation of DIARECT's biotinylated antigens a bead-based ELISA was performed using magnetic beads (Dynabeads™ M-280 Streptavidin; Thermo Fisher Scientific) coupled with biotinylated antigens. Coupling was performed according to manufacturer's recommendations with suitable modifications made. The standard ELISA protocol using HRP-conjugated secondary antibodies was adapted to the use of magnetic beads as surface material which was effectively washed by magnetic separation. Optical density (OD) was determined using a standard plate reader.

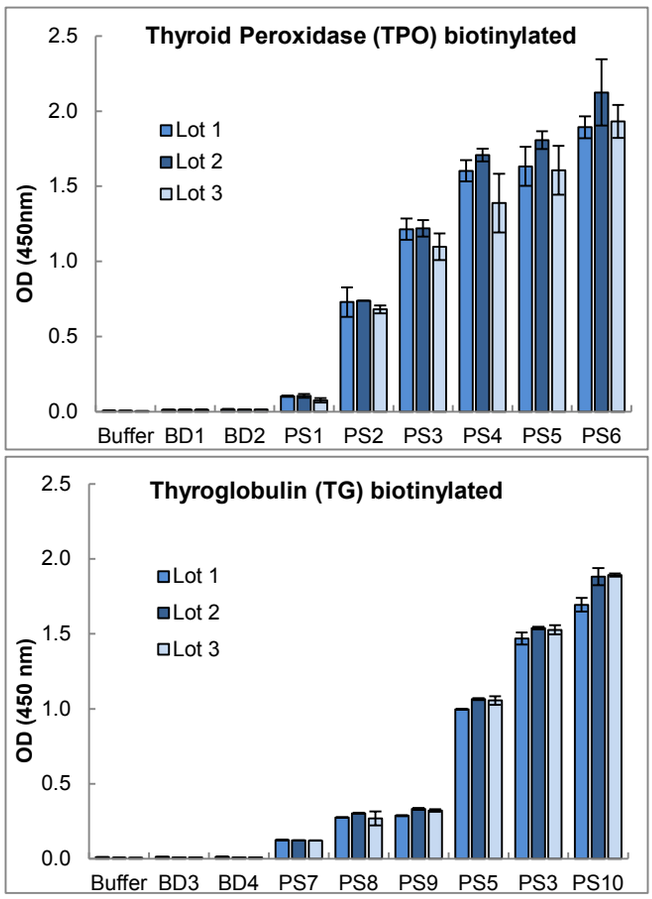

Figure: Three independent lots of TPO (upper graph) and TG (lower graph) were biotinylated and coupled onto streptavidin-coated microspheres. IgG specific responses to the antigens were detected from healthy donor (BD 1 – 4) and patient samples (PS 1 – 10), respectively (double determination). Buffer and non-biotinylated antigens (data available on request) were included as negative controls.

Ordering Information

20300 20301	Ro/SS-A (60 kDa; rec.) biotinylated	50 µg 0.5 mg
20400 20401	Ro/SS-A (60 kDa; non rec.; bov.) biotinylated	50 µg 0.5 mg
20500 20501	Ro/SS-A (52 kDa) biotinylated	50 µg 0.5 mg
20600 20601	La/SS-B biotinylated	50 µg 0.5 mg
20700 20701	GBM biotinylated	50 µg 0.5 mg
20800 20801	GAD65 biotinylated	50 µg 0.5 mg
20100 20101	Thyroid Peroxidase (TPO) biotinylated	50 µg 0.5 mg
20200 20201	Thyroglobulin (non rec.) biotinylated	50 µg 0.5 mg

References:
Weber *et al.* (1989) Science 243: 85–88
WHO (2006) Environmental Health Criteria 236: 1–333

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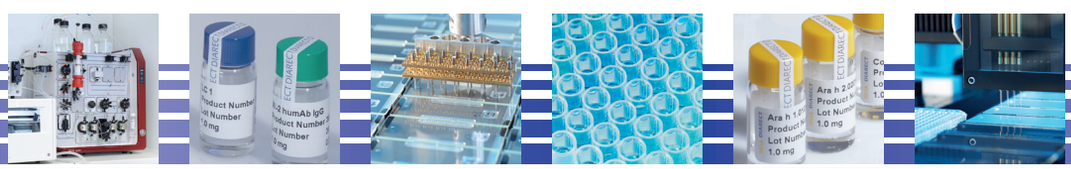

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