

sciFLEXARRAYER Application Note
No. 08002

Peptide microarrays for epitope mapping of antibodies against hTSHR

In order to use antibodies as diagnostic markers for the prediction of both prognosis and therapeutic response, accurate characterization of their antigen binding region is indispensable. To this end, comprehensive but differentiated immunoassays need to be developed. Using a peptide microarray for the diagnosis and epitope mapping of human anti-thyrotropin receptor (hTSHR) antibodies, epitopes of seven commercially available murine monoclonal antibodies specific for hTSHR (mTSHRABs) were mapped. The peptide microarray exhibited excellent performance in single and multiplex antibody analysis as well as high specificity. This technology may have potential as a multi-determinate in vitro diagnostic assay for the differential analysis of a heterogeneity of antibodies involved in the pathogenesis of autoimmune diseases

Materials and methods

A library of 251 synthetic peptides representing the primary sequence of hTSHR was site-specifically immobilized in a two-step procedure first by coupling of biotinylated peptides to hydrazide-modified streptavidin and then utilizing a subsequent chemoselective reaction between the hydrazide linkers of the streptavidin and an aldehyde-coated glass surface. Solutions were printed onto glass slides with aldehyde surface coating (Schott Nexterion® AG, Jena, Germany) with the sciFLEXARRAYER.

Results and discussion

A peptide microarray has been designed and developed that serves as a miniaturized and comprehensive tool for epitope mapping of antibody populations (Fig. 1). The patterns are highly reproducible in different arrays across the slide. The functionality of the hTSHR peptide microarray immunoassay was validated in single and multiplex analysis that demonstrated the unique capacity of the peptide microarray for simultaneous detection and accurate epitope characterization of antibody analytes.

The presented technology provides a basis for the construction of microarray immunoassays with synthetic peptides as versatile probe molecules. The immobilization technique meets the requirements for functional surface display of biologically active peptides in microspot immunoassays, especially site specificity, high probe density and stability of the linkage.

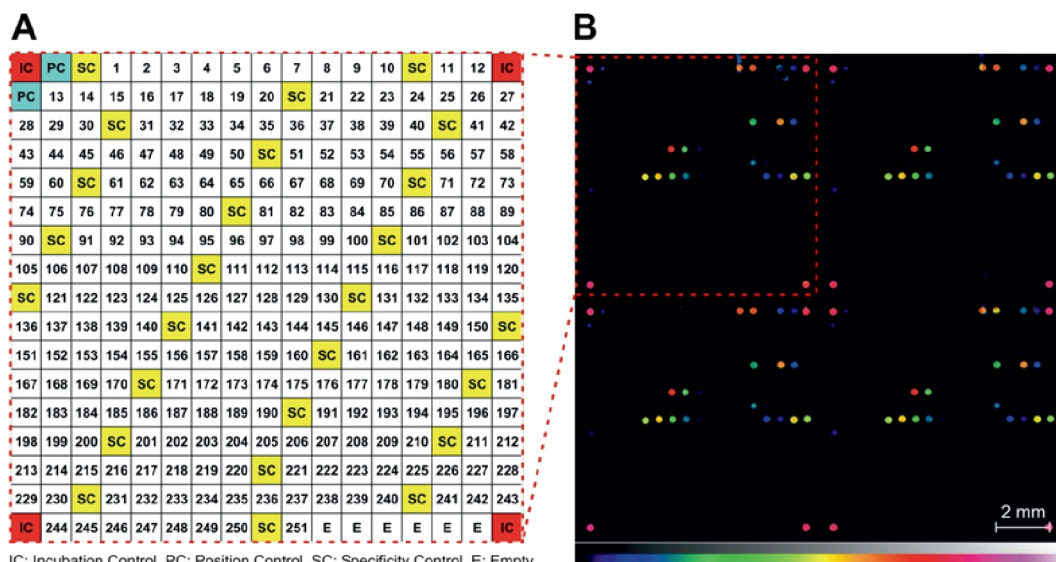


Fig. 1 Layout (A) and fluorescence image in pseudo-coloration (B) of the multiplex analysis of seven murine anti-hTSHR antibodies with the peptide microarray. The image shows a section with four identical arrays. Incubation with a mixture of mTSHRABs overnight was followed by visualisation of captured mTSHRABs with a Cy5TM-labeled secondary antibody and analysis with a GenePix® Professional 4200A microarray scanner.

Courtesy of Heiko Andresen. An article on this topic has been published by Andresen, H. et al. (2006) Development of peptide microarrays for epitope mapping of antibodies against the human TSH receptor; Journal of Immunological Methods 315, 11 – 18.

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