Gyrolab Technology

Gyrolab is a new, biosatellite platform which is designed for miniaturized immunos assay and performed in disposable CDs containing microstructures in which samples are analyzed in parallel. Each microstructure contains a 15 column of streptavidin coated particles on which reactions take place. This arrangement allows the simultaneous analysis of multiple samples using different assay formats, with each assay format having its own set of reagents and instructions. The microstructures are designed to allow for the capture and detection of specific target molecules while minimizing cross-contamination between samples. This is achieved by the use of microfluidic channels which separate different assay reagents and reactions. The microstructures are also designed to allow for the addition of reagents and substrates, as well as for the removal of waste products, in a controlled manner. This allows for the precise control of the reaction conditions and the efficient use of reagents and substrates. The use of disposable CDs also allows for easy disposal of waste products and the rapid disposal of the microstructure after use.

Experimental

Results

Selection of antibodies and assay conditions

Optimization of the assay for MabSelect SuRe ligand followed the experimental order below:

1. Selection of antibodies based on assay performance in absence of IgG
2. Investigation of assay performance in relation to assay pH
3. Selection of antibodies based on assay performance in presence of IgG

The efficiency of capturing MabSelect SuRe ligand in different environments using different pH is shown in Fig. 3. The assay for MabSelect SuRe at pH 3.5 was significantly affected at pH below 3.2. Thus it was concluded to perform the assay for MabSelect SuRe at pH 3.5. A pH lower than what is normally used for elution of IgG from chromatographic resin was used. Therefore the assay for MabSelect SuRe ligand in presence of IgG was evaluated at pH 3.5. For reference the MabSelect SuRe ligand is analyzed in absence of IgG. The assay for MabSelect SuRe ligand was successfully developed and validated for use in the Gyrolab system.

Fig. 2. Sandwich-based immunoassay miniaturized and integrated into a CD microstructure.

Conclusions

A prototype assay for MabSelect SuRe ligand in presence of excess amounts of IgG has been developed on Gyrolab.

Standards and samples are pretreated by acid dissociation for 15 min prior to analysis and the capture of MabSelect SuRe ligand is performed under acid conditions.

The assay for MabSelect SuRe operates at pH 3.5 at an IgG concentration of up to 0.6 g/L and a reaction volume of 15 nl.

Evaluation of assay performance including recovery on real process related samples as well as evaluation of different species of recombinant therapeutic IgG remain to be determined.

References


Figure 1. Sandwich-based immunoassay miniaturized and integrated into a CD microstructure.

Quantification of MabSelect SuRe ligand in presence of excess amounts of IgG on Gyrolab

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Sample preparation

Standards were prepared in two steps by mixing constant amounts of monomeric IgG at neutral pH with varying concentrations of protein A ligand ranging from 0.1 to 15ng/ml. All final IgG concentration of 0.6 g/L. Prior to analysis, samples were diluted 1:4 in 0.9 M NaCl buffers at different pH ranging from 3.2-7.6 to generate standard curves at the selected pH containing constant amounts of IgG (0.6 g/L) and varying amounts of protein A ligand (0.01-30 ng/ml).

IgG

A commercially available recombinant human monoclonal IgG (product No 10000) was purchased from Polysoratin Immunologische Forschung GmbH (Vienna, Austria).

Gyrolab Method

During the initial phase of assay development the importance of maintaining the selected pH throughout washing steps of the capture column prior to protein A ligand capture became evident. Thus several wash steps using buffers at the selected acid pH was added in the method to prevent eluting of trace amounts of IgG remaining in the upper part of the microstructure before neutralizing pH. The final method is comprised of six separate additions of liquid in which a block of acid washes is introduced prior to and after the capture step of MabSelect SuRe ligand. The final design of the method is described in Fig. 5.

Fig. 7. Illustration of factors impacting performance of MabSelect SuRe assay

Assay performance at acid and in presence of IgG

Optimally, under the conditions selected, the assay for MabSelect SuRe ligand should be unaffected by presence of IgG in samples. In experiments aimed at evaluating the effect of monoclonal IgG on the performance of MabSelect SuRe ligand assay in Bioaffy 1000, a standard curve containing constant amounts of human IgG at 0.6 g/L was analyzed. For comparison MabSelect SuRe ligand was also analyzed in absence of IgG (Fig. 6). Despite the acid conditions used, the assay is influenced by presence of IgG reducing assay sensitivity by approximately one order of magnitude. The assay capacity of detecting MabSelect SuRe ligand in presence of IgG is approximately 1 ppm (w/w).

Fig. 8. Effect of various acid and IgG concentrations on the performance of MabSelect SuRe assay.

Summary of assay conditions

A principal illustration of the assay conditions selected in relation to immuno- reagent performance and accessibility of MabSelect SuRe ligand is in Fig. 7.

Fig. 9. Illustration of factors impacting performance of MabSelect SuRe assay