

Determination of low antibody concentrations using the ConSense® analyzer

David Tschachojan, Thomas Kirner and Jörg Ackermann

Abstract

flUIT Biosystems has developed a new approach called accurate stochastic fluorescence spectroscopy (ASFS) to detect fluorescence-labeled molecules in very low concentrations in homogenous assay formats. Using the confocal ConSense® Analyzer concentrations in the picomolar range can be determined.

Method

The ASFS method is able to distinguish and quantify freely diffusing fluorescent molecules in solution. It makes use of the fact that the individual molecular properties (e.g. size, diffusion, velocity, quenching and brightness) are reflected in different sets of molecule specific parameters. The assays are carried out in simple and homogenous mix-and-measure formats.

In this application note we demonstrate the potential of ASFS for the detection of low concentrations of antibodies for therapeutic and diagnostic research applications.

Measurement and instrument settings

The graph shows the results of a dilution series of an Alexa532-labeled goat antibody (supplied by Invitrogen) in PBS standard buffer covering four orders of magnitude. The error bars indicate the standard deviation of three different series performed at different days.

Measurement parameters:

- sample volume: 3µl
- measuring time: 1 minute
- excitation power: 50 µW (532nm)
- bintime: 50µs

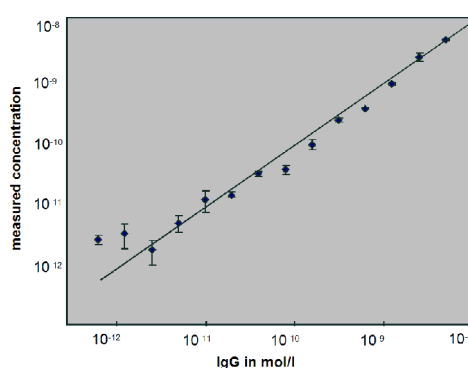


Fig 1: Measured vs. calculated Alexa532-labeled goat antibody concentration

Measurements were conducted in fluit Biosystems' 14-channel standard glass detection chips.

Conclusion

The ConSense Analyzer is able to detect and reliably quantify antibodies at nano- to picomolar levels.

For the example presented here this leads to a detection limit of 0,0015 µg/ml for an antibody of a size of 150KDa.

The ConSense Analyzer shows excellent linearity over four orders of magnitude and reproducibility of measurements.