

# Detection and Quantitation of Proteins Using a Label-free Direct Immunoassay

- Real-time, label-free protein quantitation • More informative assay development
- Reduces reagent development • Speeds analyses • Eliminates label-associated costs

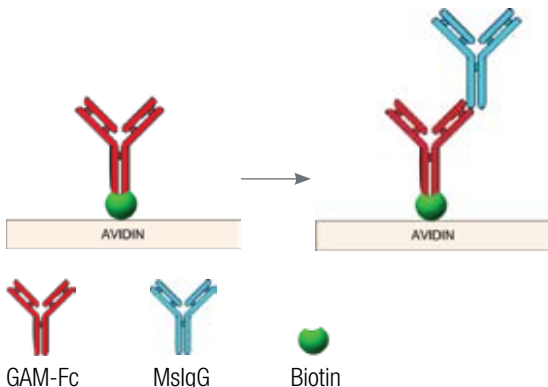


Figure 1. Immunoassay Schematic.

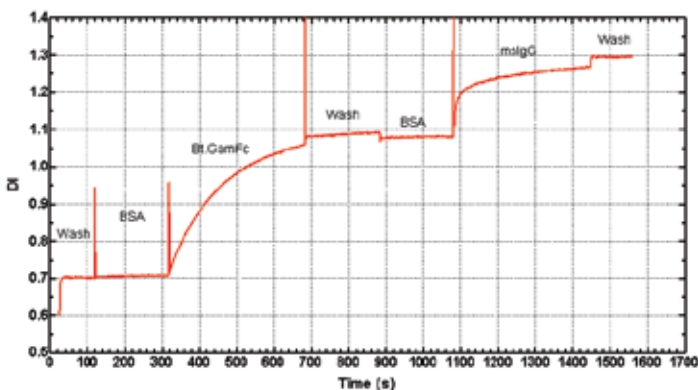


Figure 2: Real-time Data Trace. Binding of biotinylated GAM-Fc (2 µg/mL) and subsequent capture of MslgG (200 µg/mL).

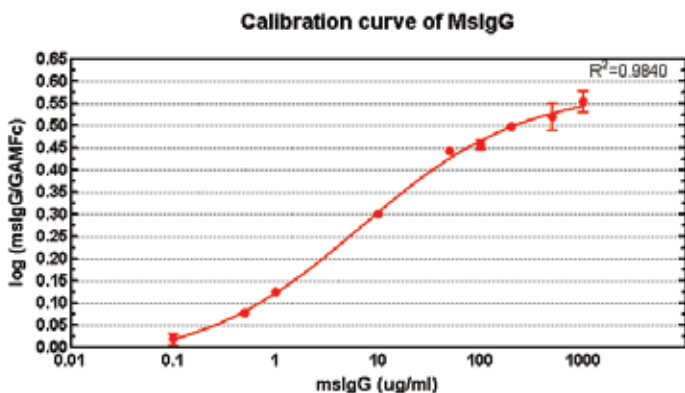


Figure 3: Calibration Curve of MslgG Bound to GAM-Fc. Standard deviations are represented by the error bars for the respective replicate assays.

## Introduction

Direct quantitation of protein analytes is essential data in many fields of research. In this study, avidin dotLab® Sensors with GAM-Fc were used to rapidly develop an assay and calibration curve for the quantitation of mouse immunoglobulin (MslgG). This assay format has general applicability for the determination of protein analytes in the nanomolar to micromolar concentration range.

## Method

For all assays, 60 µL of biotinylated goat anti-mouse Fc (GAM-Fc) (2 µg/mL) was loaded onto the avidin dot-Lab Sensor to generate a specifically oriented anti-mouse capture surface. An equivalent volume of MslgG (positive control) at concentrations of 0.5, 1.0, 10, 50, 100, 200, 500 and 1000 µg/mL then were introduced. A schematic of the complexes formed is shown in Figure 1. A blocking solution of Bovine Serum Albumin (BSA) (5 mg/mL) in PBS-T was loaded before each step.

## Results

- Figure 2 shows direct, real-time detection of: 1) the PBS-T wash, 2) BSA blocking, 3) biotinylated GAM-Fc binding to the avidin sensor surface, 4) a second PBS-T wash, and 5) a BSA blocking step prior to the introduction of MslgG.
- The calibration curve was generated using varying concentrations of MslgG. Ratios of the MslgG binding signal to the GAM-Fc binding signal (measured in DI) were determined, and plotted versus MslgG concentration.
- Figure 3 shows these results fitted to a sigmoidal dose response curve. A sample with an unknown quantity of MslgG can now be compared against the curve to obtain a concentration estimate.
- The limit of detection for this assay and reagents is 0.2 µg/mL as defined by the value of two standard deviations over the average for the zero determination.

## Conclusion

This direct, label-free dotLab System application can be used for quantitative determination of nanomolar and micromolar level analytes. This particular MslgG example may be useful in monitoring the recovery of monoclonal antibodies throughout a purification process. A similar approach can be used to quantitate high concentration analytes in a variety of matrices. The real-time binding data generated enable more immediate decisions as compared to a traditional ELISA assay, where data interpretation is based solely on end-point results. System uniquely permits the development and use of new multiplexed panels, resulting in greater analytical utility.

