

Protein A ELISA for detection of MabSelect SuRe™ ligand: Performance Comparison vs. Other Commercially Available Kits

Introduction

Immobilized Protein A provides a convenient method for the purification of antibodies, both on the small and large scale. Small amounts of Protein A leached from the purification resin may contaminate purified antibody samples. Repligen has developed a new ELISA Kit specifically for quantitating residual MabSelect SuRe™ ligand (P/N 9333-1). This Protein A construct is the least similar to other commercially available Protein A ligands and is not accurately quantitated when using some other commercially available Protein A ELISA Kits. Repligen's kit provides the most accurate, precise, and sensitive measure of MabSelect SuRe™ ligand.

Materials & Methods

For this comparison four commercial Protein A ELISA Kits were evaluated:

- Repligen's Protein A ELISA for the detection of MabSelect SuRe™ ligand P/N 9333-1
- Cygnus's Protein A ELISA P/N F400
- Cygnus's Protein A ELISA P/N F050H
- AssayDessigns's Protein A EIA P/N 900-057

An independent testing contractor ran a single kit from each manufacturer according to the manufacturer's instructions. Three independent preparations of MabSelect SuRe™ ligand Test Sample were prepared and ran in an 8-point dilution series. The dilution series spanned the range of the standard curve. Each dilution was assayed in triplicate.

Results

Repligen's Protein A ELISA is Highly Precise

Table 1. Coefficient of Variation was calculated for non-zero points of standard curve and MabSelect SuRe™ ligand sample. All concentrations were tested in triplicate. All calculated values for a kit were less than the number reported.

Kit	CV (%)
Repligen Protein A ELISA for MabSelect SuRe™ ligand	<4.0 %
Kit 2	< 13.0 %
Kit 3	< 11.0 %
Kit 4	< 21.0 %

Repligen's Protein A ELISA is Highly Accurate

Table 2. Recovery of MabSelect SuRe™ ligand from three independent preparations runs in triplicate across multiple dilutions.

Kit	% Recovery ± SD
Repligen Protein A ELISA for MabSelect SuRe™ ligand	119.2 ± 9.5
Kit 2	181.1 ± 22.6
Kit 3	67.8 ± 20.2
Kit 4	12.4 ± 0.8

MabSelect SuRe™ is a trademark of GE Healthcare companies.

Recovery of MabSelect SuRe™ ligand

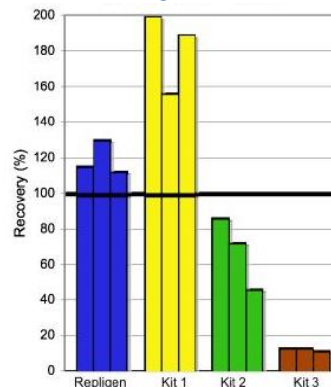


Figure 1. Recovery of MabSelect SuRe™ sample from four commercially available Protein A ELISA Kits. Each bar represents recovery of a separate preparation of sample (Preparation A, B, C). Recovery is determined as the ratio of calculated/nominal concentration, as a percent.

Repligen's Protein A ELISA has Dilution Linearity

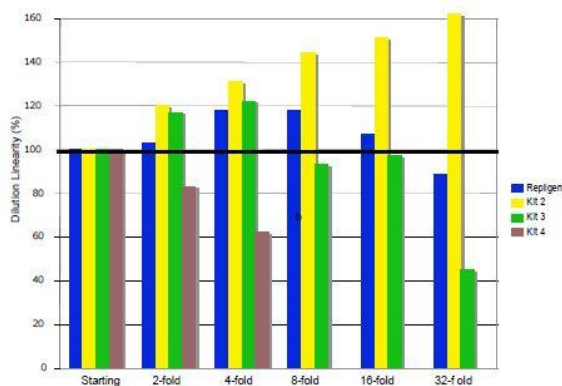


Figure 2. Dilution Linearity of commercially available Protein A ELISA Kits. A single preparation of MabSelect SuRe™ ligand was 2-fold serially diluted across 6 concentrations and assayed in triplicate. The highest concentration was set as 100% and recovery for all remaining concentrations determined as a percent. Kit 4 absorbance values dropped to background levels at the 8-fold dilution point.

Conclusions

Results confirm that Repligen's Protein A ELISA for detection of MabSelect SuRe™ ligand offers:

- The most accurate quantitation of the MabSelect SuRe™ ligand compared to other commercially available kits tested.
- The highest precision in replicate wells compared to other kits.
- The most consistent dilution linearity at low concentrations, ensuring sensitivity at low protein levels.

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