Quantitation of MabSelect SuRe™ Protein A Ligand: Why a Matched Standard Matters

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Introduction

Immobilized Protein A resins are commonly used in the purification of monoclonal antibodies and fusion proteins since they achieve greater than 90% purification starting from complex feed streams. The success of Protein A chromatography has resulted in a variety of affinity resins, which are manufactured using several different Protein A variants. There are four major Protein A variants used in common affinity resins: recombinant Protein A (Repligen), MabSelect™ ligand (GE Healthcare), native Protein A (Millipore) and MabSelect SuRe™ ligand (GE Healthcare).

Repligen offers two ELISA Kits for quantitation of residual Protein A. The MabSelect SuRe™ Protein A ELISA Kit (part number, 9333-1) is designed exclusively for detection of the MabSelect SuRe™ ligand, while the Recombinant Protein A ELISA Kit (part number 9000-1) is recommended for all other Protein A variants. This technical note describes why a unique ELISA kit with a matched ligand standard is required to accurately quantitate leached MabSelect SuRe™ ligand.

Protein A Variants

Native Protein A is expressed on the surface of Staphyloccocus aureus and consists of five immunoglobulin binding domains (designated E, D, A, B, and C), a tail domain (designated as X),and a transmembrane domain. Other variants are recombinant and are expressed in E. coil. Three of the four variants contain all five binding domains whereas MabSelect SuRe™ ligand is a tetramer of a modified domain which has been designated as the Z domain. Table 1 shows domain structure and molecular weight for the variants. The large difference in molecular weight between MabSelect SuRe™ ligand and the other variants creates a need for a matched standard.

Table 1. Protein A Characteristics

Name	Domain Structure	Molecular weight*
Recombinant Protein A	EDABCX	44618
Native Protein A	EDABCX	46760
MabSelect™ ligand	EDABC	34317
MabSelect SuRe™ ligand	ZZZZ	26747

Methods

Protein A variants were obtained from the respective suppliers. Solutions were prepared at 1.0 mg/mL in water and stored frozen until use. ELISAs were performed following ELISA Kit instructions. Each Protein A variant was assayed at concentrations from 0.05 to 1.6 ng/mL in triplicate wells over three assays. Each assay contained all four variants on the same ELISA plate.

Data Analysis

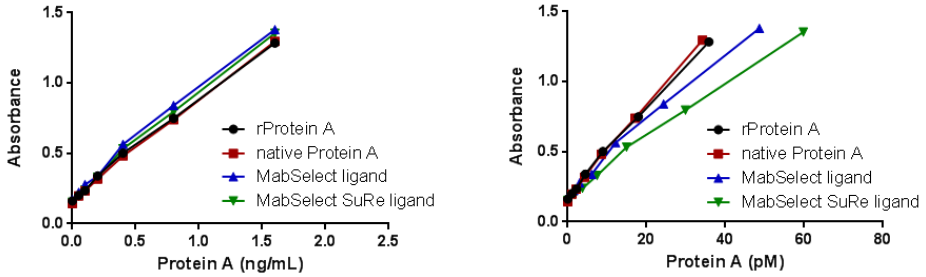
Standard curves were graphed as Absorbance versus Concentration, in either ng/mL or pM units. It was necessary to convert to pM values to account for differences in molecular weight of the Protein A variants. Recoveries (i.e. accuracy) were calculated for each variant against the recombinant Protein A standard curve. Recovery was defined as the calculated concentration divided by nominal, expressed as a percent. A recovery of 100% implies accurate quantitation of the input Protein A. High, medium, and low concentrations were assessed, defined as 1.6, 0.4, and 0.1 ng/mL respectively. Recoveries were also calculated using concentration reported in molarity units where the molecular weight of each Protein A variant was first used to convert its concentration in ng/mL to pM.

Results and Discussion

ELISA results are commonly graphed as Absorbance versus concentration. Figure 1A presents a representative graph of Protein A (ng/mL) versus Absorbance from one of the three assays. When concentration is plotted as ng/mL the four variants have similar responses. However, MabSelect SuRe™ ligand is approximately 2-fold lower in molecular weight and therefore graphing as molar concentration gives greater accuracy. Figure 1B illustrates the same data plotted as Protein A (pM) versus Absorbance.

Figure 1. Absorbance response as a function of Protein A Variant Concentration

(A) Protein A variants expressed as ng/mL (B) Protein A variants expressed a pM



Inaccuracy when using an unmatched standard can be further illustrated by calculating recoveries. Recoveries were calculated for high, medium, and low concentrations of the different variants using recombinant Protein A as the standard. Table 2 shows recoveries calculated from ng/mL concentrations of each variant while Table 3 presents recoveries when concentrations are in pM units.

Each value is the average from three separate assays.

Table 2. Recoveries (in %) of Protein A variant calculated using ng/mL concentrations

	Recovery:		
Concentration:	Native Protein A	MabSelect™ ligand	MabSelect SuRe™ ligand
High	89.9	105.7	98.7
Medium	103.8	129.7	114.8
Low	67.0	111.1	78.7

Table 3. Recoveries (in %) of Protein A variant calculated using pM concentrations

	Recovery:		
Concentration:	Native Protein A	MabSelect™ ligand	MabSelect SuRe™ ligand
High	94.2	77.9	59.2
Medium	108.8	96.7	68.8
Low	70.2	82.0	47.2

If an ELISA were run quantitating MabSelect SuRe™ ligand against an unmatched standard such as recombinant Protein A it would appear that the calculated concentration is accurate, as shown in the right most column of Table 2. However, weight-based comparisons are only valid when the two species being compared have the same molecular weight. Calculating recoveries in molar units, as in Table 3, shows that the calculated concentration is inaccurate and in fact substantially under-estimates the amount of MabSelect SuRe™ ligand present. The FDA requires quantitation because residual Protein A can be a patient safety risk. Therefore, increased risk results from under-estimating the amount of Protein A present in a sample.

Summary

The success of Protein A chromatography has resulted in the creation of a number of Protein A variants. Although these all function to bind immunoglobulin they are unique molecules with different characteristics. The lower molecular weight of MabSelect SuRe™ ligand leads to under-reporting of concentration if an unmatched standard is used and results are reported in ng/mL.

To accurately quantitate leached MabSelect SuRe™ ligand, Repligen's ELISA Kit for the detection of MabSelect SuRe™ is the only commercially available kit which uses a matched reference standard to deliver the most accurate, precise, and sensitive measurements.