Evaluation of five commercial immunoassays for quantification of leached ligand from a new protein A resin

Intellectual Property Notice: The Biopharma business of GE Healthcare was acquired by Danaher on 31 March 2020 and now operates under the Cytiva™ brand. Certain collateral materials (such as application notes, scientific posters, and white papers) were created prior to the Danaher acquisition and contain various GE owned trademarks and font designs. In order to maintain the familiarity of those materials for long-serving customers and to preserve the integrity of those scientific documents, those GE owned trademarks and font designs remain in place, it being specifically acknowledged by Danaher and the Cytiva business that GE owns such GE trademarks and font designs.

CY14404-12un20-PT

Evaluation of five commercial immunoassays for quantification of leached ligand from a new protein A resin

Elin Monie, Annika Forss, and Tomas Björkman
GE Healthcare Bio-Sciences AB, Björkgatan 30, 75184 Uppsala, Sweden

Abstract

There are several advantages with protein A affinity purification of monoclonal antibodies, for example, high robustness, selectivity, and purity. However, one side effect of protein A chromatography is that variable amount of protein A ligand is eluted together with the product, and there is a regulatory demand to control and quantify the protein A amounts throughout the purification process. A new protein A chromatography resin, MabSelect™ PrismA, has been developed based on a new high-flow agarose base matrix in combination with a new alkaline-stabilized protein A ligand. The key features of the resin include increased binding capacity and the possibility to use up to 1 M sodium hydroxide for cleaning in place and sanitization. Here, five different commercially available protein A immunoassays were evaluated for detection and quantification of the new protein A ligand. In the commercial kits, different variants of protein A are included as standard. A comparison between the different assays in respect to quantification using existing standard and the new ligand was made. A comparison of ligand standard curve quality and dynamic range as well as experimental procedures was also made.

Results

Abcam kit:

- Similar shapes for the two standard curves but different responses (Fig 1A).
- The dynamic range is about one log.
- The MabSelect PrismA ligand standard gives higher concentrations compared with kit standard (Fig 1B).

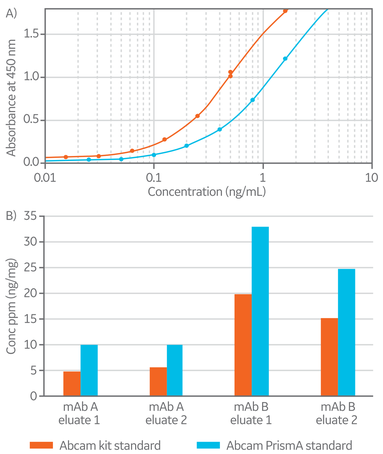

Fig 1. (A) Comparison of MabSelect PrismA and Abcam kit standard curves. (B) Quantification of samples, using both Abcam kit standard and MabSelect PrismA standard.

Cygnus kit:

- The MabSelect PrismA standard gives lower response than the kit standard.
- For the MabSelect PrismA standard, additional standard points with higher concentration (15 and 20 ng/mL) were required (Fig 2A).
- The dynamic range is about one log.
- The MabSelect PrismA standard gives higher concentrations compared with kit standard (Fig 2B).

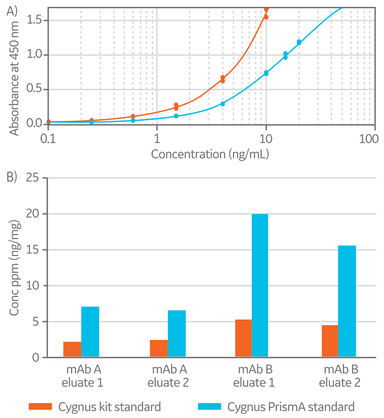

Fig 2. (A) Comparison of MabSelect PrismA and Cygnus kit standard curves. (B) Quantification of samples, using both Cygnus kit standard and MabSelect PrismA standard.

Genscript kit:

- Similar shapes for the two standard curves but different responses (Fig 3A).
- The dynamic range is about one log.
- The MabSelect PrismA standard gives lower concentrations than kit standard (Fig 3B).

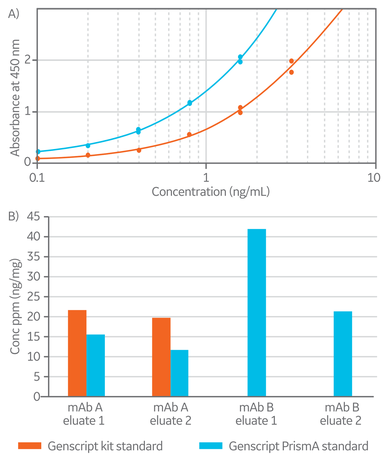

Fig 3. (A) Comparison of MabSelect PrismA and Genscript kit standard curves. (B) Quantification of samples, using both Genscript kit standard and MabSelect PrismA standard. No results obtained for mAb B with kit standard.

Gyrolab™ kit:

- Similar shapes for the two standard curves but different responses (Fig 4A).
- The dynamic range is about three logs.
- The MabSelect PrismA standard gives lower concentrations than kit standard (Fig 4B).

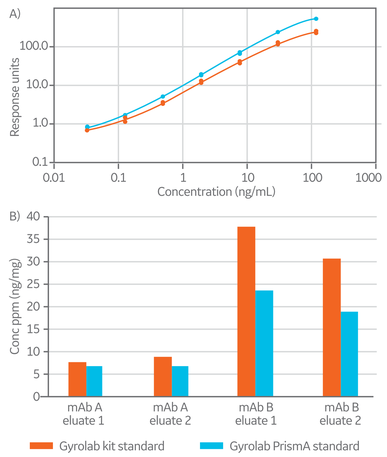

Fig 4. (A) Comparison of MabSelect PrismA and Gyrolab kit standard curves. (B) Quantification of samples, using both Gyrolab kit standard and MabSelect PrismA standard.

Repligen kit:

- Similar shapes for the two standard curves but different responses (Fig 5A).
- The dynamic range is about one log.
- The MabSelect PrismA standard gives higher concentrations than kit standard (Fig 5B).

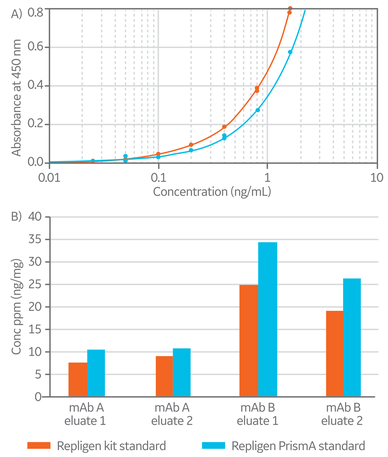

Fig 5. (A) Comparison of MabSelect PrismA and Repligen kit standard curves. (B) Quantification of samples, using both Repligen kit standard and MabSelect PrismA standard.

Experimental

The tested immunoassays are summarized in Table 1. The assays were performed according to kit protocol.

Two MabSelect PrismA eluates from two different monoclonal antibodies, mAb A and mAb B, were used as test samples. Both MabSelect PrismA standard and respective kit standard were used in the assays.

Table 1. Summary of tested immunoassays

	Abcam protein A ELISA kit (ab133036)*	Cygnus protein A Assay (#F400)*	Genscript protein A ELISA Kit (L00430)†	Gyrolab protein A kit (P0020456)†	Repligen protein A ELISA Kit (9000-1)†
Pretreatment for dissociation of protein complex	Heat	Heat	Heat	Acidic, fully automatic	Acidic
Same treatment for samples and standards	Yes	Yes	No, only samples are heat treated	Yes	Yes
Time for sample preparation (h)	1.5 (dilution, pretreatment)	1.5 (dilution, pretreatment)	1 (dilution, pretreatment)	1 (dilution)	1 (dilution, pretreatment)
No. of incubation steps	4	2	4	None, automatic procedure	4
Incubation temp. (°C)	RT	RT	37	No incubation	RT
Shake incubation	Yes	Yes	No	No	No
Estimated procedure time (h)	4.5	4	3	2.5–3	2.5
Kit standard curve range (ng/mL)	0–1	0–10	0–3.2	0–120	0–1.6
Extra equipment needed	Microplate reader	Microplate reader	Microplate reader	Gyrolab workstation	Microplate reader

* rProtein A included in kit used as standard. † MabSelect SuRe™ ligand used as standard in kit.

Conclusions

- MabSelect PrismA standard curve could be obtained in all five immunoassays tested.
- It was possible to quantify MabSelect PrismA samples with all five immunoassays (Fig 6).
- MabSelect PrismA samples could be quantified using the kit standards, but MabSelect PrismA standard should be used for a better estimation of the quantity.
- The MabSelect PrismA standard gives in general higher concentrations compared with rProtein A kit standard.
- Gyrolab protein A kit has the largest dynamic range with three logs in comparison to the other kits with one log.
- A comparison between the different assays in respect to quantification using existing standard and the new ligand was made. A comparison of ligand standard curve quality and dynamic range as well as experimental procedures was also made.

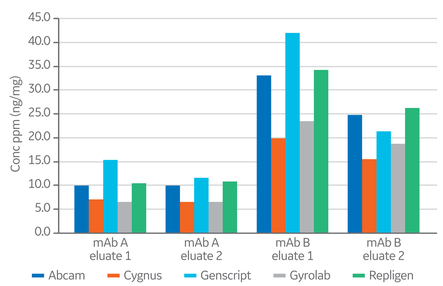

Fig 6. Quantification of MabSelect PrismA samples using MabSelect PrismA standard in five different protein A immunoassays.

gelifesciences.com/bioprocess

KA2425022818PO