

Residual Protein A for Bioprocessing Applications

Introduction

Most therapeutic monoclonal antibodies (mAbs) are isolated from cell culture media using Protein A chromatography¹. During purification, Protein A can leach from the solid phase, ending up in the purified product. This can be problematic as regulatory guidelines dictate that detection and reduction of residual Protein A are necessary to obviate potential immunogenic consequences in vivo². Thus, there is pressing need to rapidly detect residual Protein A contamination during product characterization with high sensitivity and throughput. ELISA-based methods are time-consuming and labor-intensive, requiring extensive hands-on setup, followed by manual washing and incubation steps at carefully timed intervals. Furthermore, the sensitivity of ELISA varies depending on the antibody and protein antigen, and may not be suitable to detect very low levels of protein (<1 ng/mL). These shortcomings are compounded by matrix interference effects that often confound ELISA results.

ProteinSimple's Wes™ provides a powerful solution by bringing immunoassay into a capillary electrophoresis format. Its operation is fully automated, requiring no user intervention after a simple setup, thereby greatly reducing time and labor while increasing precision and reproducibility. It minimizes matrix interference effects so you can be confident the quantification of Protein A in your sample is accurate. In addition, you gain molecular weight information of your Protein A contaminant. Here, we show you how Wes can quickly detect Protein A with high sensitivity, down to 28 pg/mL, without all the caveats of an ELISA.

Materials

ProteinSimple

- Instrument: Wes
- Wes 12–230 kDa Separation Module (PN SM-W004)
- No Secondary Detection Module (PN DM-003)

Protein A reagents

- Recombinant Protein A short form (Novus Biologicals, NBP2-34964)

- Recombinant Protein A long form (Novus Biologicals, NBP2-34966)
- Anti-Protein A antibody (R&D Systems, MAB99761)

Other reagents

- Donkey anti-Rat HRP (Jackson ImmunoResearch, 712-035-153)
- CHO HCP Antigen Concentrate, 3G (Cygnus Technologies, F553X)

Methods

1. Prepare 0.1X Sample Buffer by diluting the 10X Sample Buffer 1:100 in water.
2. Dilute the anti-Protein A antibody to 1.2 µg/mL in Antibody Diluent 2 (PN 042-203).
3. Dilute the Donkey anti-Rat HRP secondary antibody to 13.5 µg/mL in Wes Antibody Diluent 2.
4. To determine the LOD and LOQ, reconstitute the short and long forms of recombinant Protein A antigens per the manufacturer's recommendations and then create a serial dilution series of the antigens in 0.1X Sample Buffer.
In this example, a 2-fold dilution series from 400 pg/mL to 25 pg/mL was created.
5. To determine the LOD and LOQ in the presence of CHO HCP, dilute the CHO HCP stock in 0.1X Sample Buffer to 0.1 mg/mL and then create a serial dilution series of the antigens in 0.1X Sample Buffer and 0.1 mg/mL CHO HCP.
6. Follow the default Wes sample preparation and assay conditions.

Results

To establish the assay range, titrations of the Protein A short and long forms were evaluated with Wes. **Figure 1** shows the electropherograms of the Protein A antigen titrations, **Figure 2** shows the linearity of the Protein A antigen titrations, and **Figure 3** shows the LOD and LOQ.

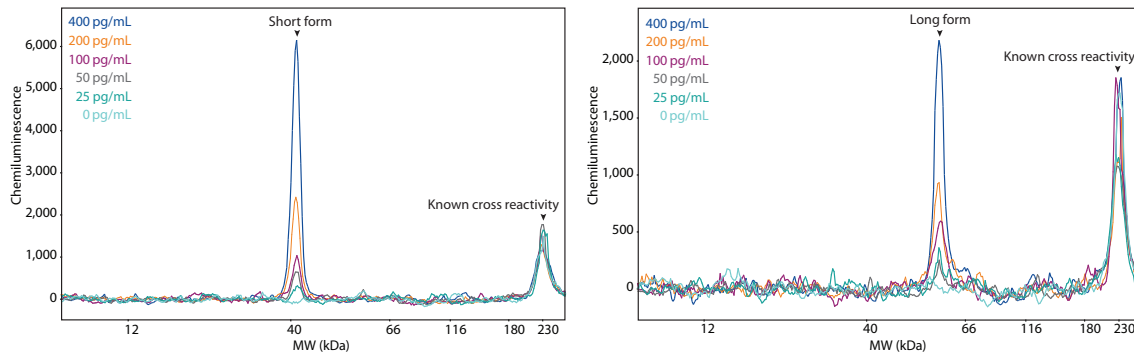


FIGURE 1. Protein A detection with Wes. Protein A short form (left) and long form (right) were diluted into 0.1X sample buffer and titrated from 400 pg/mL to 25 pg/mL. Protein A was detected using an anti-Protein A antibody.

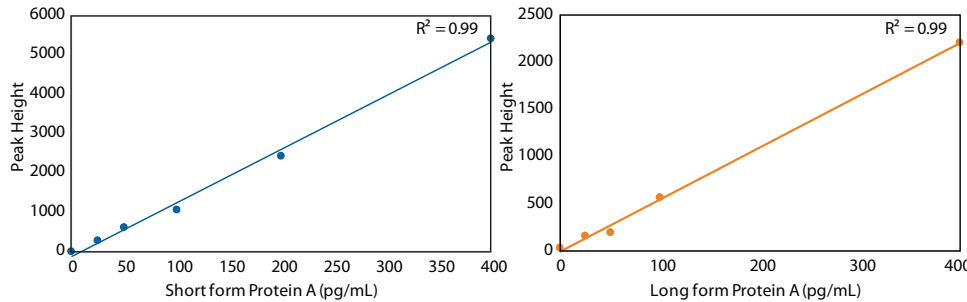


FIGURE 2. Linearity of the Protein A antigen titrations. Protein A short form (blue) and long form (orange) were diluted into 0.1X sample buffer and titrated from 400 pg/mL to 25 pg/mL. Protein A was detected using an anti-Protein A antibody.

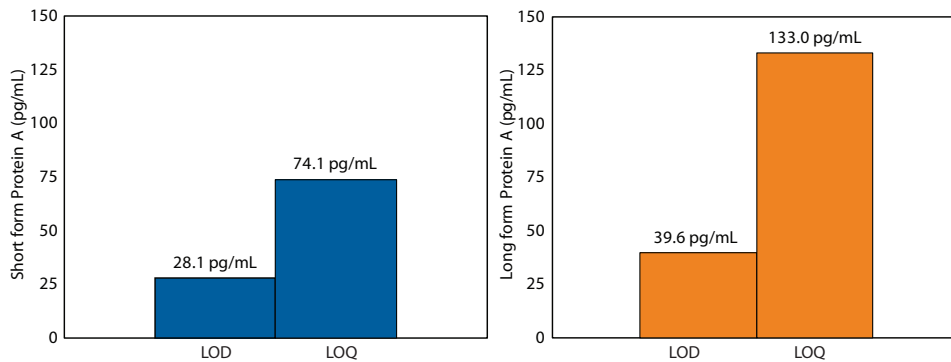


FIGURE 3. LOD/LOQ measurements for Protein A antigens. Protein A short form (blue) and long form (orange) LOD/LOQ's were determined using linearity data shown in Figure 2.

The Protein A antibody shows slight cross reactivity with the 230 kDa internal fluorescent standard. This binding will not impact quantitation as Protein A short and long forms have apparent molecular weights of ~42 and ~55 kDa and are well-separated from the 230 kDa peak. To avoid this cross reactivity, 2–40 kDa fluorescent master mix (Fluorescent 5x Master Mix 5, PN PS-FL05-8) may be used in place of the standard 12–230 kDa master mix.

To reflect a real-world scenario, the Protein A short form was spiked into a lysate background. We used the Cygnus CHO HCP Antigen Concentrate (0.1 mg/mL) and compared the LOD/LOQ in the absence and presence of CHO HCP lysate (Figure 4). The linearity comparison shows a minor change in detection of Protein A in the presence of CHO HCP. Additionally, the LOD/LOQ for Protein A increased in the presence of CHO HCP.

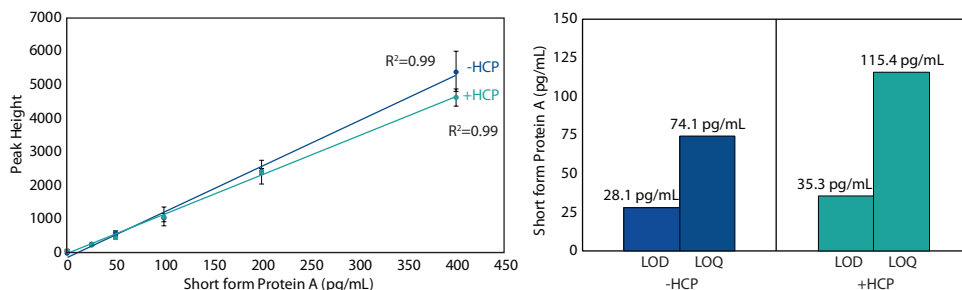


FIGURE 4. Linearity (left) and LOD/LOQ data (right) of the Protein A in CHO HCP. A titration of short form Protein A was performed in a consistent amount of CHO HCP control antigen (0.1 mg/mL) and compared to Protein A diluted in 0.1 X sample buffer. Linearity data (left) show only a minor change in the slope of the Protein A detection. The limit of detection and LOQ (right) increased slightly in the presence of CHO HCP antigen.

Conclusion

Using off-the-shelf antibodies and default Wes settings, we developed a quick and robust assay to detect Protein A. Unlike ELISA, no user intervention is required after initial setup, and sensitivity can be achieved that is comparable to or surpasses ELISA-based methods. As little as 28.1 pg/mL was detected, and even in the dense and complex CHO HCP lysate solution, a lower limit of detection of Protein A was achieved at 35.3 pg/mL. For these reasons, Simple Western makes a powerful solution to streamline workflows to meet regulatory standards in the product development pipeline of monoclonal antibodies.

References

1. Protein A chromatography for antibody purification, S Hober, K Nord and M Linhult, *Journal of Chromatography B*, 2007; 848:40-47.
2. Points to consider in the manufacture and testing of monoclonal antibody products for human use, U.S. Food and Drug Administration, 1997.



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