Optimize Your icIEF Fractionation Method on the MauriceFlex System

with Simple Western pI Markers

Introduction

The MauriceFlex™ system enables icIEF fractionation of protein charge variants, and it allows for collecting 24 to 84 fractions on a 96-well plate. The identity and purity of these collected fractions then need to be verified by analytical icIEF, which can be time-consuming if all fraction wells need to be analyzed.

To reduce the number of fraction wells for verification, the range of the fraction wells that contain the charge variants should be precisely located and selected for subsequent verification. One approach is to use the peak prediction feature in the Compass for iCE software. The prediction algorithm is based on tracking the mobilization of the charge variants inside the capillary imaging window and extrapolating their "arriving time" onto the 96-well plate. The peak prediction generally works well for regular mAbs (like NIST mAb) but may function incorrectly for mAbs with more complex or irregular charge profiles, and will cease to work (disabled) if re-focusing is used.

The other approach is to use a "read from top" fluorescence plate reader, where the measured fluorescence is used to identify wells that may contain charge variants. However, the fluorescence plate reader typically only detects charge variant fractions of high concentrations, especially the main peak, but does not have enough sensitivity to locate the low abundance acidic and basic charge variant fractions. As a result, additional fractions from both acidic and basic sides are needed for verification to cover a complete distribution of charge variants.

In this technical note, we describe an alternative approach to use a fluorescence plate reader for locating the range of fraction wells of protein charge variant fractions. In this approach, Simple Western (SW) pI markers (**Table 01**) are spiked into the sample loaded onto the fractionation cartridge.

TABLE // 01

List of Simple Western and Maurice pI markers used in this study

Catalog Number	Item
040-024	SW pI Marker 4.0
040-025	SW pI Marker 4.2
040-026	SW pI Marker 4.4
040-027	SW pI Marker 4.9
040-028	SW pI Marker 5.5
040-029	SW pI Marker 6.0
040-030	SW pI Marker 6.4
040-031	SW pI Marker 7.0
040-032	SW pI Marker 7.3
041-036	SW pI Marker 8.4
040-790	SW pI Marker 9.7
046-028	Maurice pI Marker 3.38
046-029	Maurice pI Marker 4.05
046-030	Maurice pI Marker 5.85
046-031	Maurice pI Marker 6.14
046-032	Maurice pI Marker 7.05
046-033	Maurice pI Marker 8.40
046-047	Maurice pI Marker 9.99
046-035	Maurice pI Marker 10.17

These SW pI markers do not emit natural fluorescence, therefore appearing “transparent” and thus will not interfere with the natural fluorescence detection during the MauriceFlex fractionation (**Figure 01**). However, when the plate is subjected to excitation on the plate reader (excitation/emission: 550/580 nm), the fraction wells containing the eluted SW pI markers will emit strong fluorescence, providing references for easy identification of any protein charge variant fractions with pI values between these pI markers.

FIGURE // 01

Maurice icIEF electropherogram of Simple Western pI Markers

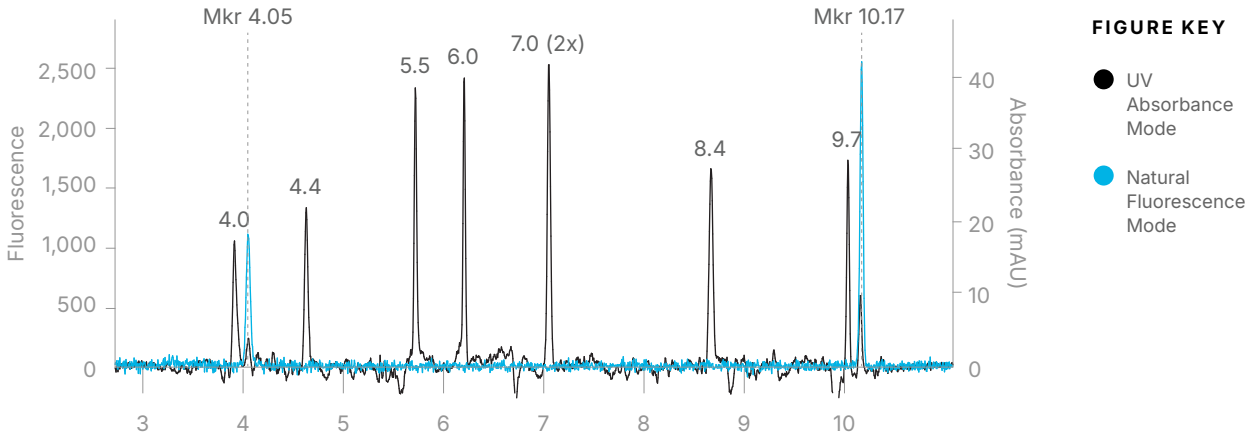

Figure 01. Maurice icIEF electropherogram of Simple Western pI markers in UV absorbance mode (black) and natural fluorescence mode (blue). Minimal natural fluorescence from the SW pI markers. The “Mkr 4.05” and “Mkr 10.17” are the two Maurice pI markers used for icIEF.

Results

Here we demonstrate the approach with USP mAb2 (**Figure 02A**). Two SW pI markers (pI 6.0 and pI 8.4), bracketing the pI values of the charge variants, were added to the Maurice pI marker mix prior to fractionation. The pI marker mix is made of equal volumes of two usual bracketing Maurice icIEF markers (pI 6.14 and 8.4) and the two additional bracketing SW pI markers. This mix was added to the master mix for a final concentration of 0.5% per pI marker (i.e., 2 µL marker mix in 100 µL of master mix).

The fractionation of USP mAb2 on MauriceFlex with the above master mix was set to collect 36 fractions on a 96-well plate, followed by checking on the fluorescence plate reader (SpectraMax® i3x Multi-Mode Microplate Reader by Molecular Devices). **Figure 02B** shows a plot of the fluorescence signal intensities of the 36 fractions on the plate. As shown, the fraction of two SW pI markers (C12 and C1) emitted significantly stronger fluorescence signals, much like “flag posts” compared to other fractions, and between them are the 10 fractions (C11 to C2) that contain the charge variants **Figure 02B**, green bars). Therefore, only these 10 fractions, instead of all 36 fractions, needed to be verified. Subsequent icIEF analytical analysis confirmed that the USP mAb2 charge variants were between C9 to C6 (**Figure 02C**).

FIGURE // 02

SW pI markers enable easy identification of charge variant fractions of USP mAbs

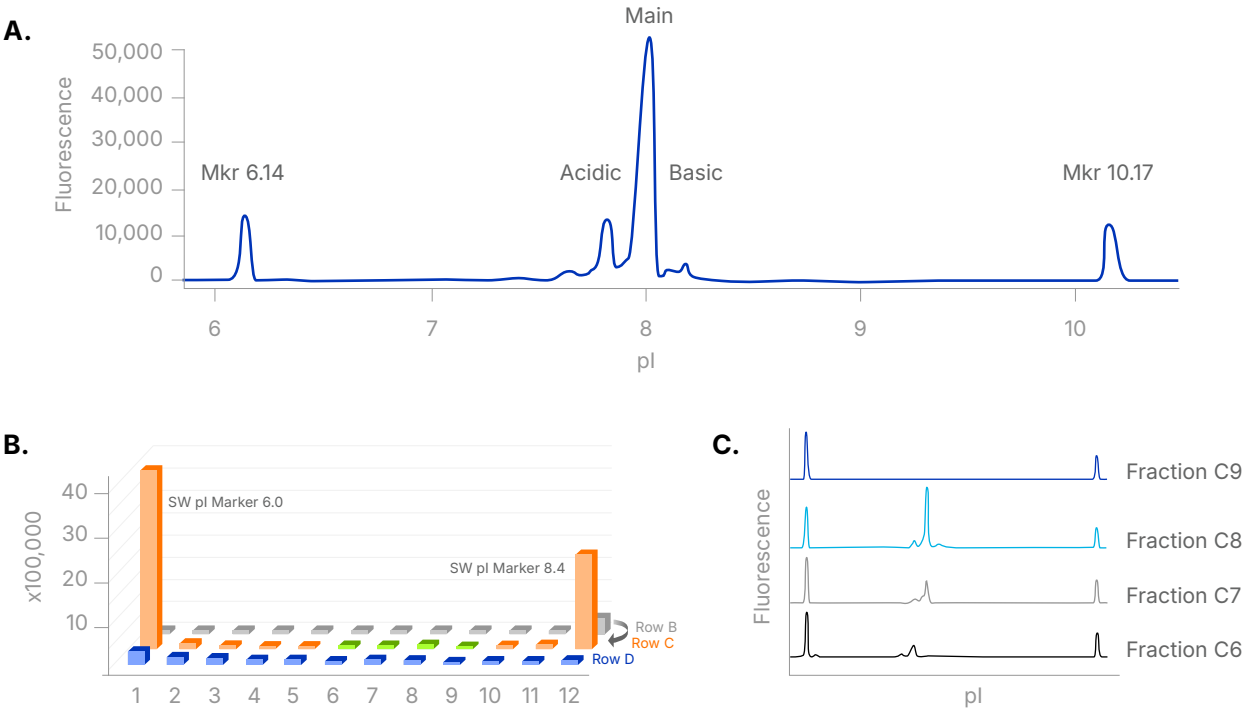

Figure 02. SW pI markers enable easy identification of charge variant fractions of USP mAbs. A. icIEF electropherogram of USP mAbs on Maurice; B. 3D plot of fluorescence intensities of 36 fractions on a plate reader. Note the fractions were collected following B1 to B12, then C12 to C1, then D1 to D12. The green fractions are the charge variant fractions confirmed by Maurice icIEF; C. Maurice icIEF verification results of fractions of charge variants of C9 to C6.

Conclusion

This approach is easy to implement and effective in locating the range of fractions containing charge variants when refocusing is used and peak prediction is disabled. By carefully choosing the supplemental SW pI markers to bracket the protein charge variants to be fractioned, a narrower range of fraction wells can be selected for verification, thus saving time in the overall MauriceFlex fractionation workflow for charge variant analysis.

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