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Application Note

Simplify the Qualification of Your GMP Proteins with Maurice™

Introduction

The quality of your cell therapy product directly depends on the quality of raw materials and reagents used during its manufacturing. For example, certain cytokines and interleukins, also classified as "ancillary materials" (AMs), are routinely used for cell expansion, activation, and proliferation[1,2]. Being of biological origin, these proteins are highly prone to variability and therefore require that their quality be carefully controlled for optimal performance. While AMs are typically manufactured under Good Manufacturing Practice (GMP) guidelines, the responsibility of qualifying these AMs rests with the cell therapy manufacturer. Quality attributes of AMs such as identity, purity, functionality, and others are evaluated during qualification. Some of these attributes are qualified using laborious techniques such as SDS-PAGE, which is a lengthy analytical method with many challenges. Because of these hurdles, there is a need for alternative robust analytical methods that address those challenges and deliver reliable data.

This application note describes a fast, easy, and streamlined method for characterizing the identity and purity of three commonly used GMP cytokines in cell therapy manufacturing—IL-2, IL-7, and IL-10—using Maurice, an automated capillary electrophoresis (CE) instrument for icIEF (charge) and CE-SDS (size) analysis.

Both icIEF and CE-SDS methods can be used on a single Maurice instrument by simply switching between their respective pre-assembled cartridges. Maurice takes care of the cleanup while ensuring that no cross-contamination takes place. Protein charge heterogeneity data is obtained between 10-15 minutes, and purity data within 5.5-35 minutes. Crucially, Compass for iCE and Waters Empower® 3 Chromatography Data software ensure that the data you get is 21CFR Part 11 compliant. Fast, reliable results, coupled with excellent reproducibility will let you develop your methods within a day.

Materials and Methods

The following GMP proteins were obtained from R&D Systems, a Bio-Techne brand: Recombinant Human IL-2 GMP Protein (PN BT-002-GMP), Recombinant Human IL-7 GMP Protein (PN BT-007-GMP), and Recombinant Human IL-10 GMP Protein (PN 1064-GMP). The Maurice cIEF Method Development Kit (PN PS-MDK01-C), Maurice CE-SDS PLUS Application Kit (PN PS-MAK03-S), and Maurice CE SDS Molecular Weight Markers (PN 046-432) were obtained from ProteinSimple, a Bio-Techne brand, and contained all the materials and reagents for icIEF and CE-SDS assays, respectively. Additionally, for CE-SDS analysis, iodoacetamide (IAM, PN I16125) and β-mercaptoethanol (β-ME, PN M-3148) were obtained from Millipore Sigma.

icIEF Methods

All samples were prepared in Maurice 96-well plates (PN 046-021) and were run on a Maurice instrument. IL-2 samples were diluted to 0.05 mg/mL with an ampholyte mixture containing Pharmalytes 3-10 (4%), urea (5.5M), ASB-14 (0.5%), and arginine (5 mM). IL-7 samples were diluted to 0.1mg/mL with an ampholyte solution containing Pharmalytes 3-10 (4%) and SimpleSol (50 µL). IL-10 samples were also diluted to 0.1 mg/mL with an ampholyte solution containing Pharmalytes 3-10 (4%) and urea (4M). pI markers 5.85 and 9.99 were added to all three GMP cytokine samples. All samples were separated at 1500 V for 1 minute and then 3000 V for 10 minutes. For detection, absorbance and native fluorescence modes were used and data were analyzed using Compass for iCE software.

CE-SDS Methods

IL-2, IL-7, and IL-10 samples were each diluted to 0.1 mg/mL with CE-SDS PLUS 1X Sample Buffer and were treated with either IAM (12.5 mM) for non-reduced analysis or β-ME (710 mM) for reduced analysis. All samples were denatured at 70°C for 10 minutes, after which they were transferred to Maurice 96-well plates. All samples were injected for 20 seconds at 4600 V and separated for 25 minutes at 5750 V. Data were analyzed using Compass for iCE software.

Results

IL-2

Three lots of IL-2 samples were evaluated using icIEF and CE-SDS. **FIGURE 1** shows an overlay of the icIEF native fluorescence profiles for all three lots, demonstrating excellent consistency.

FIGURES 2A and **2B** show the purity profiles obtained from non-reduced and reduced CE-SDS, respectively. Each dataset shows an overlay of profiles of all three lots and includes data from the "Lane View" feature of Compass for iCE, which can be leveraged to view results similar to the format of traditional SDS-PAGE.

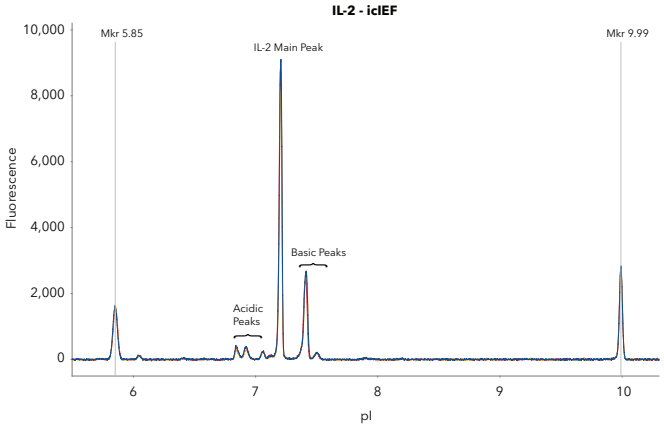

FIGURE 1. icIEF analysis of three lots of IL-2. An overlay of charge profiles from three sample lots are shown.

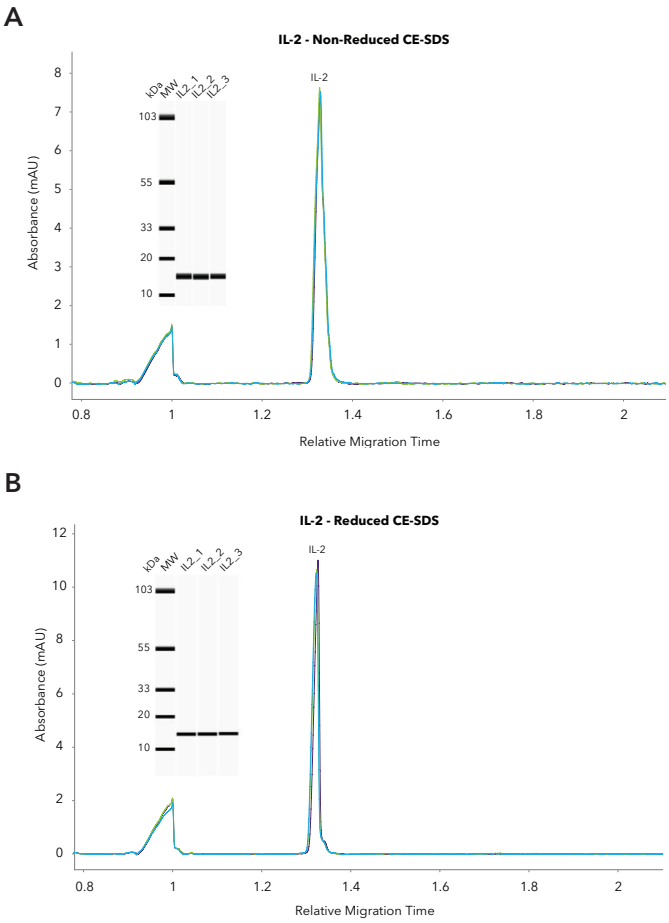

FIGURE 2. CE-SDS analysis of IL-2. A. An overlay of electropherograms obtained from three different IL-2 lots under non- reduced conditions and B. under reduced conditions. The traditional gel-like insets to the right of each figure are from the "Lane View" feature in Compass for iCE software. Both datasets show excellent lot-to-lot reproducibility.

IL-7

FIGURE 3 shows the charge profiles of three consecutive sample injections of IL-7, detected with native fluorescence. **FIGURES 4A** and **4B** show the results from non-reduced and reduced CE-SDS analysis, respectively, which again are overlays of three consecutive sample injections. Both CE methods resulted in outstanding reproducibility.

IL-10

FIGURE 5 shows the results from icIEF analysis of IL-10 samples, also detected with native fluorescence. The samples were run using the same experimental conditions as IL-7, and the results show perfectly overlaid electropherograms generated from three consecutive sample injections. Similarly, **FIGURES 6A** and **6B** each show overlays of three sample injections from non-reduced and reduced CE-SDS analyses, respectively. As observed for IL-2 and IL-7, the results obtained for IL-10 were also highly reproducible.

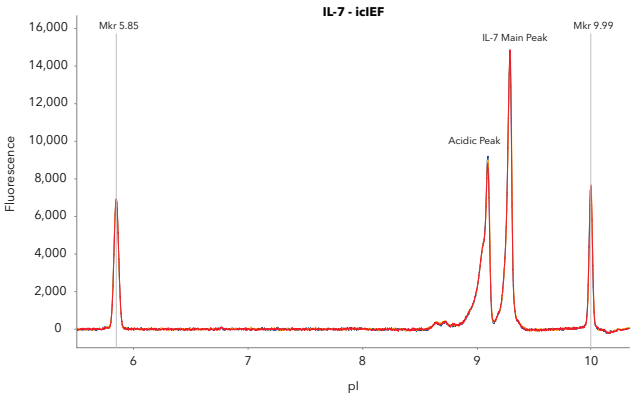

FIGURE 3. icIEF analysis of three consecutive IL-7 sample injections with native fluorescence detection.

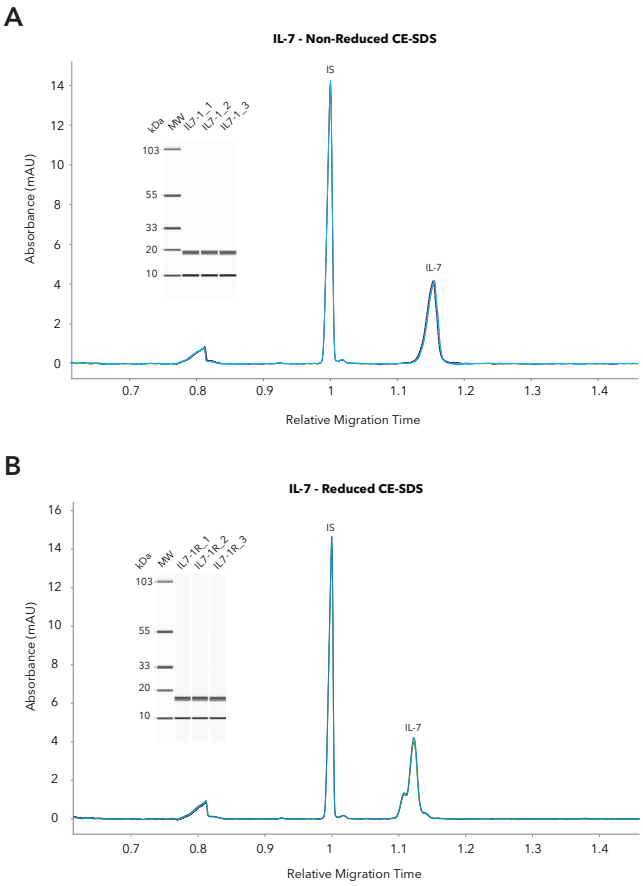

FIGURE 4. CE-SDS analysis of IL-7. **A.** An overlay of three sample injections run under non-reduced conditions and **B.** reduced conditions, with insets representing a gel-like view. IS: Internal Standard.

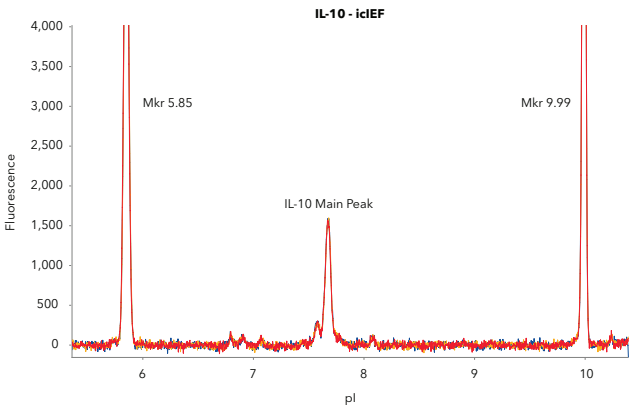

FIGURE 5. icIEF analysis of three consecutive IL-10 sample injections with native fluorescence detection.

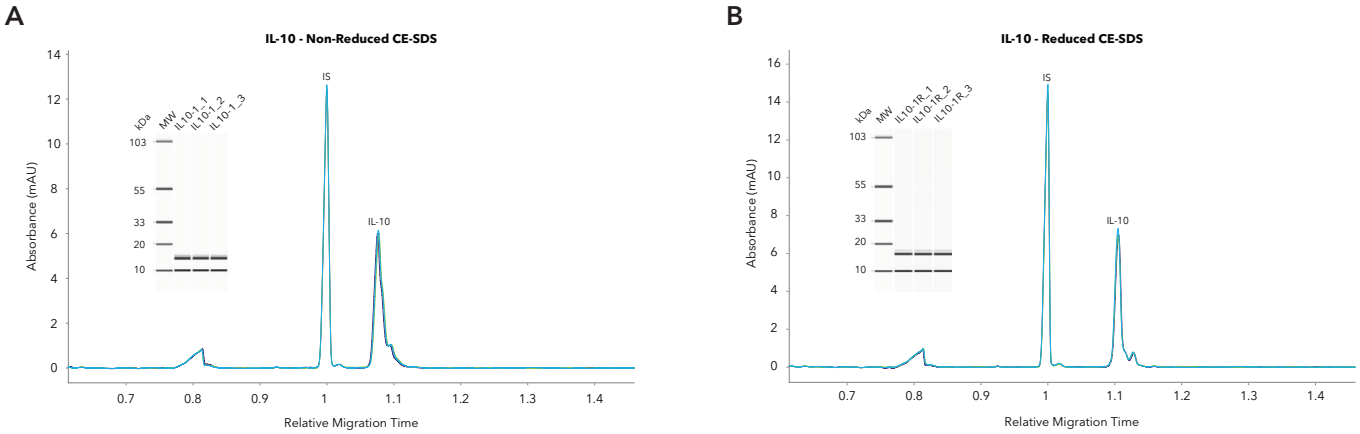

FIGURE 6. CE-SDS analysis of IL-10. A. An overlay of three sample injections run under non-reduced conditions and B. under reduced conditions. Results from the Lane View feature are also included. IS: Internal Standard.

Conclusion

Today, the best-in-class GMP proteins and other AMs from Bio-Techne are readily available for use in cell therapy manufacturing. However, to be sure of their quality, robust and reliable analytical methods are key for both the vendor as well as the cell therapy manufacturer. The good news is that such methods can be remarkably easy! This application note has described the identity and purity characterization of three commonly used animal-free GMP proteins—IL-2, IL-7, and IL-10—using the Maurice instrument, which is well known in the industry for its ease-of-use, reproducibility, and high-quality data for CE analysis. Using a single instrument, both charge and size profiles were obtained for all three proteins from Bio-Techne, demonstrating outstanding lot-to-lot and method reproducibility.

This data highlights the quality of the GMP proteins as well as the reliability of analytical methods with Maurice. Furthermore, the ease with which this data was generated is not only a reflection of a simplified workflow, but also promises a seamless transfer across different stages, from development to QC.

The Maurice system serves as a one-stop CE solution for not just GMP proteins, but for several other molecules such as monoclonal antibodies, biosimilars, ADCs, and viral vectors. No matter which of these molecules you're working on, rest assured that Maurice will significantly accelerate your drug development. Visit us to learn more about Maurice or about our GMP proteins.

References

1. United States Pharmacopeia (2022). *General Chapter, ⟨1043⟩ Ancillary Materials for Cell, Gene, and Tissue-Engineered Products.* USP-NF. Rockville, MD: United States Pharmacopeia https://doi.org/10.31003/USPNF_M620_02_01

2. Decot, V., Voillard, L., Latger-Cannard, V., Aissi-Rothé, L., Perrier, P., Stoltz, J. F., & Bensoussan, D. (2010). Natural-killer cell amplification for adoptive leukemia relapse immunotherapy: comparison of three cytokines, IL-2, IL-15, or IL-7 and impact on NKG2D, KIR2DL1, and KIR2DL2 expression. *Experimental hematology*, *38*(5), 351–362. https://doi.org/10.1016/j.exphem.2010.02.006