Advanced Electrophoresis Solutions Ltd.

Product Fact Sheets and Catalog

2025

ADVANCED
ELECTROPHORESIS SOLUTIONS LTD.

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Introduction

icIEF is a powerful tool for characterizing protein charge heterogeneity, and in particular, has become essential for monitoring the quality of monoclonal antibodies (mAbs). However, the icIEF landscape is rapidly changing:

a) There is a growing need to understand, in great detail, the modifications underlying each charge variant, as this information can help avoid costly delays during the late stages of development and manufacturing.

b) Emerging modalities like ADCs, fusion proteins, and AAVs have demonstrated great potential, but their complexity challenges the limits of existing charge variant characterization methods.

c) A heightened awareness of instrument discontinuation and critical reagent shortages have highlighted the need for a greater selection of icIEF vendors.

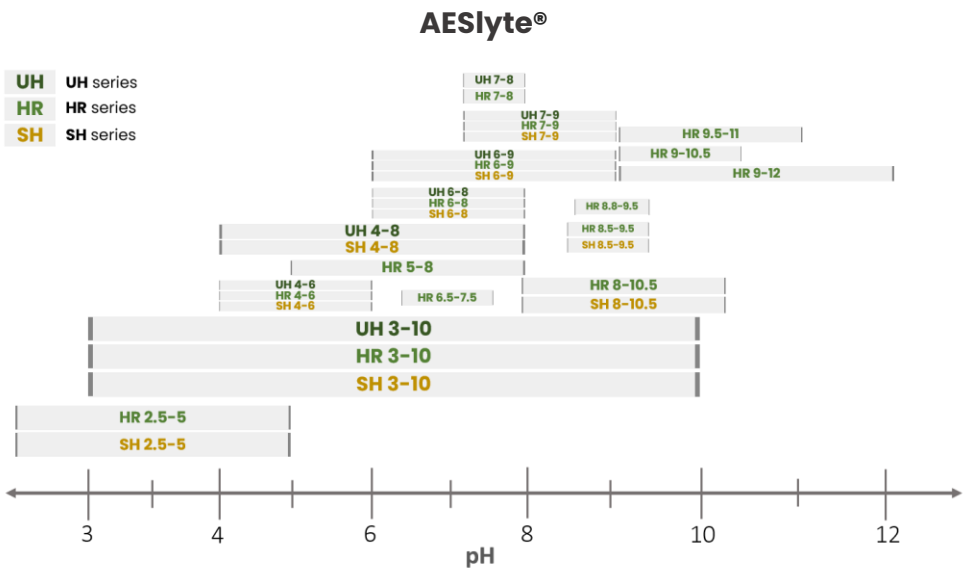
In response to these industry demands, many companies have been reconsidering their current approach and evaluating more advanced icIEF tools. Throughout this transition, AES has been actively supporting customers with various products and services:

a) CEInfinite. Run analytical and preparative icIEF with confidence, and begin to identify the modifications hidden beneath each charge variant peak (Fig 1).

b) Critical Reagents. Simplify the analysis of complex samples by leveraging the next generation of carrier ampholytes, pI markers, etc (Fig 2).

Fig 1. The CEInfinite can flexibly switch between analytical icIEF, icIEF fractionation, and icIEF-MS.

c) Advanced Solutions. AES is much more than an icIEF vendor. We are a reliable partner committed to ensuring all aspects of your icIEF operations run smoothly and consistently. With our expertise and resources, we can help you address your most urgent challenges. As biopharma increasingly relies on icIEF and technical demands grow more complex, the benefits of partnering with AES will become even more apparent.



pI Markers

2.85*	5.91*	8.79
3.21	6.14	9.22
3.38	6.61	9.33
3.59	7.00	9.46
4.05	7.03	9.50
4.10	7.05*	9.77
4.14	7.35	9.86
4.22	7.40	9.99
4.50	7.46*	10.00
4.65	7.55	10.10
5.12	7.65	10.17
5.28*	7.90	10.45
5.45*	8.18	11.20
5.50*	8.40	
5.85*	8.71	

Fig 2. There is a wide selection of both broad and narrow range carrier ampholytes. The HR, SH, and UH AESlytes® each have different molecular properties that can be leveraged to optimize icIEF resolution for specific samples. There is also a wide selection of pI markers, with the ones highlighted in green representing fluorescent molecules, and the ones marked with an asterisk (*) representing those that are resistant to digestion by the carboxypeptidase B (CpB) enzyme.

Reliable critical reagents

The Industry Challenges. icIEF is known for its simple method development. The standard master mix can be finely tuned in a day, and trusted to separate most monoclonal antibodies.

However, with the emergence of more complex modalities like bispecific antibodies, fusion proteins, antibody/drug conjugates (ADCs), adeno-associated viruses (AAVs), and lipid nanoparticles, many of the technical difficulties affecting performance have been magnified, including solubility, pH gradient resolution, capillary wall adsorption, background interference, lot-to-lot consistency, and more.

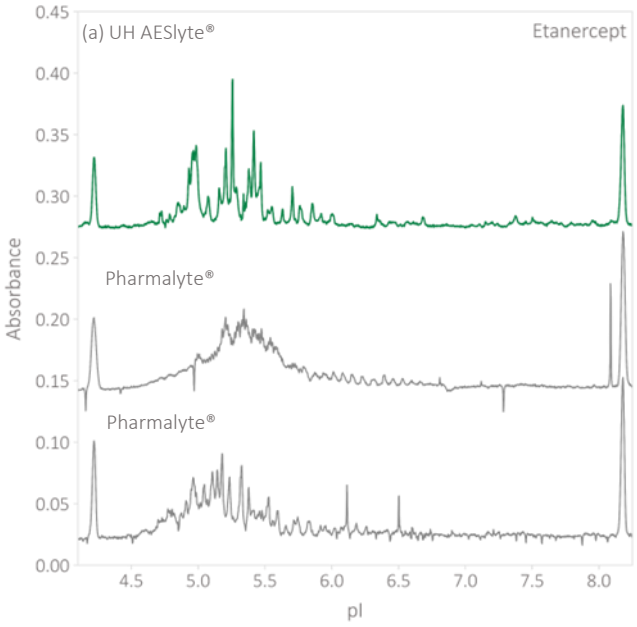
If the charge heterogeneity of these complex samples cannot be adequately resolved, or if reproducibility issues arise during quality control, this will undoubtedly delay their release. Consequently, there is an urgency to develop a wider selection of critical reagents for a more tailored approach to method development. In general, more choice would also help ease concerns related to supply chain disruptions after their vulnerability was exposed during the recent pandemic.

The Advanced Electrophoresis Solution. AES has been meeting these industry demands by leveraging a deep understanding of icIEF and developing an innovative approach to chemical design and synthesis.

→ AESlyte®. Critical reagents as ampholytes influence the resolution and reliability of icIEF, and their availability and quality are essential to the success of biopharma operations.
There are 3 different carrier ampholyte series to choose from, and each includes a variety of narrow pH ranges for increased resolution (Fig 2). The HR series delivers results similar to that of Pharmalyte® while providing outstanding lot-to-lot consistency[15,16]. The SH and UH series can be used independently or mixed with the HR series. The UH series, in particular, consists of entirely new molecular structures with unmatched resolution (Fig 3).

→ Capillary Coatings. In addition to the hydrophobic fluorocarbon (FC) coating, there are durable hydrophilic coatings made from either acrylamide derivative (AD) or methylcellulose (MC). These coatings expand the diversity of samples that can be analyzed by icIEF while eliminating the need for MC in solution, thereby avoiding issues like pre-rinse, bubble formation, and capillary clogging.[9]

→ pI Markers. There are 40+ markers ranging from 2.85 to 11.2 (Fig 2). Many are fluorescent, and several are resistant to carboxypeptidase B (CpB) enzyme digestion.



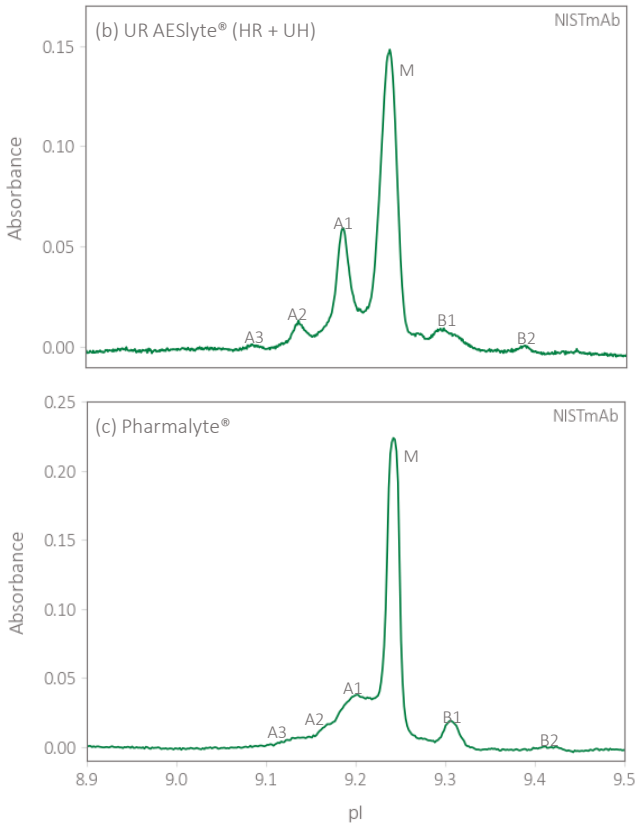


Fig 3. (a) icIEF profile for the fusion protein Etanercept using different carrier ampholytes. Note the outstanding baseline resolution for UH AESlyte®. (b) and (c) The icIEF profile using UR AESlyte® and Pharmalyte, respectively. The UR AESlyte® is a combination of HR and UH AESlyte®, and it is clear that the resolution is substantially improved.

AESlytes® and pI marker List

HR AESlyte® Part# 5 mL

2.5 → 5	101013
3 → 7	101046
3 → 10	101016
4 → 6	101040
5 → 8	101014
6 → 8	101041
6 → 9	101017
6.5 → 7.5	101048
7 → 8	101019
7 → 9	101011
7.5 → 8.5	101075
8 → 10.5	101015
8.5 → 9.5	101018
8.8 → 9.5	101042
9 → 10.5	101043
9.5 → 11	101044
9 → 12	101047

SH AESlyte® Part# 5 mL

2.5 → 5	101023
3 → 10	101026
4 → 6	101030
4 → 8	101024
6 → 8	101031
6 → 9	101027
7 → 8	101029
7 → 9	101021
8 → 10.5	101025
8 → 9	101032
8.5 → 9.5	101028

UH AESlyte® Part# 5 mL

2.5 → 5	101063
3 → 10	101066
4 → 8	101074
4 → 10	101072
4 → 5	101064
4 → 6	101070
6 → 9	101067
7 → 8	101069
7 → 9	101071
8 → 10.5	101065
8.5 → 9.5	101068

pI Markers Part# 250 uL

2.85*	100201	6.14	100213	9.33	100225
3.21	100202	6.61	100214	9.46	100226
3.38	100234	7.00	100215	9.50	100227
3.59	100203	7.03	100231	9.77	100228
4.05	100242	7.05*	100216	9.86	100239
4.10	100204	7.35	100233	9.99	100236
4.14	100205	7.40	100217	10.00	100238
4.22	100206	7.46*	100243	10.10	100229
4.50	100240	7.55	100218	10.17	100237
4.65	100207	7.65	100219	10.45	100230
5.12	100209	7.90	100220	11.20	100241
5.28*	100235	8.18	100221		
5.45*	100258	8.40	100222		
5.50*	100210	8.71	100232		
5.85*	100211	8.79	100223		
5.91*	100212	9.22	100224		

Green = fluorescent
*CpB enzyme resistant

ISO 9001 2015 CERTIFIED

Order online:
www.aeslifesicences.com

Differentiated Analytical icIEF

Overview of Capabilities for icIEF on CEInfinite

Software Control	Dedicated AES Consumables	Capability and Support
✓ Industry Standard Control via Chromeleon™ and Empower ™ available.	✓ Reproducibility Stringent lot-to-lot manufacturing controls.	✓ Preparative icIEF Upgrade for fractionation and icIEF-MS capabilities.
✓ Compliance Ready CEInfinite software enabling 21 CFR Part 11 compliance.	✓ Coated Capillaries 200+ runs for FC coating, 50+ runs for AD and MC.	✓ Reliability Simple design minimizes potential failure points.
✓ Automation 96-well plate autosampler for unattended operation.	✓ Robust Supply Chain Supply agreements available for AESlytes® and more.	✓ Long Term Support Avoid the uncertainty of technology discontinuation.

icIEF on CEInfinite for USP mAbs

(a)	Apparent Main pI			Acidic Area			Main Area			Basic Area		
	Average	SD	%RSD	Average	SD	%RSD	Average	SD	%RSD	Average	SD	%RSD
NISTmAb	9.24	0.0016	0.018	31.1%	0.48	1.56	59.9%	0.72	1.21	9.1%	0.21	2.35
USP mAb001	9.20	0.007	0.076	36.5%	0.67	1.84	56.9%	0.63	1.11	6.6%	0.23	3.50
USP mAb002	7.94	0.012	0.145	24.4%	0.17	8.05	71.3%	0.33	0.47	4.3%	0.12	6.43
USP mAb003	7.87	0.015	0.192	17.6%	0.57	6.92	63.1%	0.56	0.89	19.4%	0.20	0.65

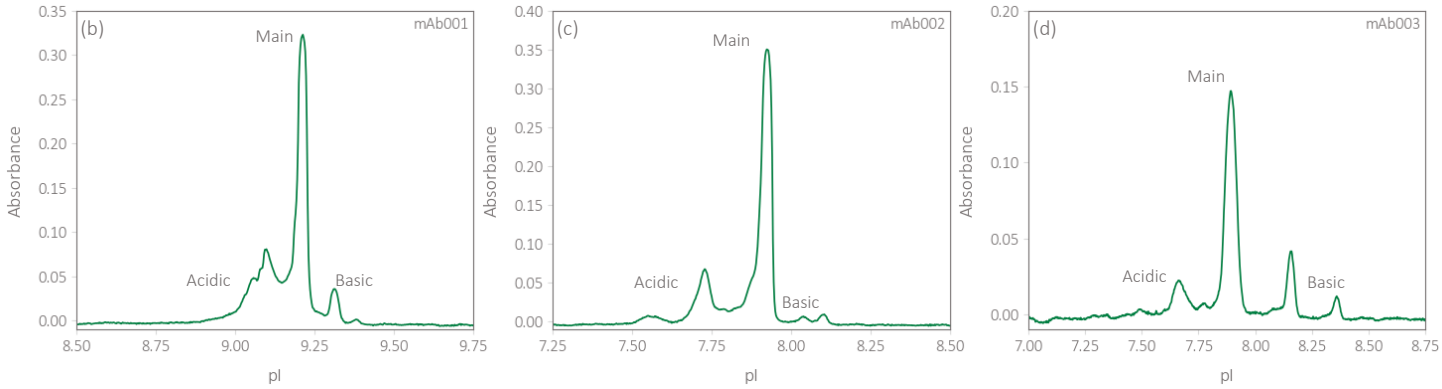
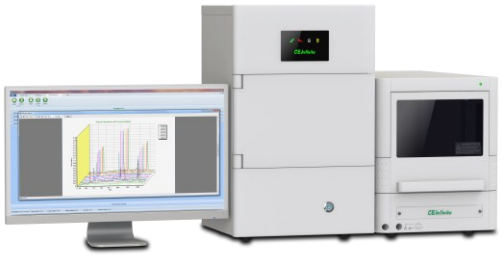


Fig 4. (a) A summary of icIEF data averaged over 6 consecutive runs with the CEInfinite for different monoclonal antibody reference standards. The methods were chosen according to similar evaluations by iCE3 and Maurice,[17-19] and the results are comparable. The corresponding icIEF profiles for the USP samples (a) mAb001, (b) mAb002, and (c) mAb003 are shown above, while the NISTmAb profile can be found in Fig 3c. Please contact AES for further information on the materials, methods, and data analysis.

Analytical icIEF model

Part# CE10002



- Seamless icIEF platform method transfer from iCE3™ and Maurice™.
- 21 CFR Part 11 compliance ready.
- GMP friendly.
- Chromeleon™ and Empower™ control ready, for QC and regulation filing.
- Life-time support.

Analytical icIEF consumables and kits

Cartridges

Part#	Coating	Info
CP00201	FC-Fluorocarbon Hydrophobic	Analytical
CP00202	FC-Fluorocarbon Hydrophobic	Analytical High sensitivity
CP00301	AD-Acrylamide derivative Hydrophilic	Analytical
CP00302	AD-Acrylamide derivative Hydrophilic	Analytical High sensitivity
CP00501	MC-Methylcellulose Very hydrophilic	Analytical

Kits

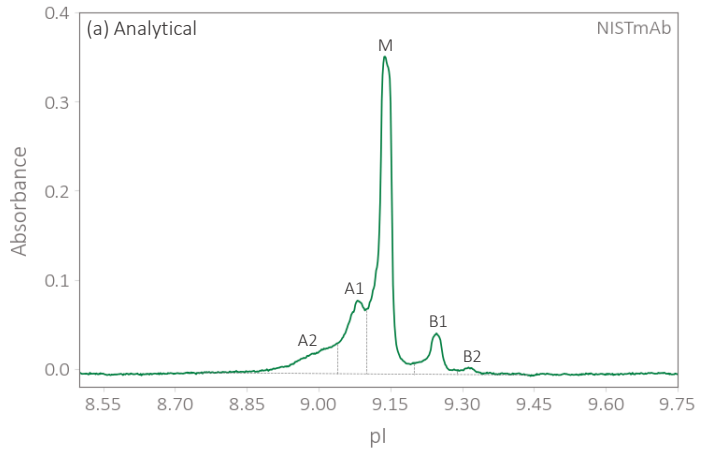
Part#	Info
101003	1.0% Methyl Cellulose (MC), 100 mL
101005	0.5% Methyl Cellulose (MC), 100 mL
201051	icIEF/CEInfinite System Suitability Kit with Hemo, for all icIEF platforms,500 uLx10.
101010	Electrolyte Solutions kit (Anolyte, Catholyte), 100 mLx2.
100051	icIEF/CEInfinite Analytical System Test Kit with 6 pI mark mixture, 25 mL.
SV00801	PP, 300uL Sample Vial, Snap Ring, 100/pack with pre-cut caps.

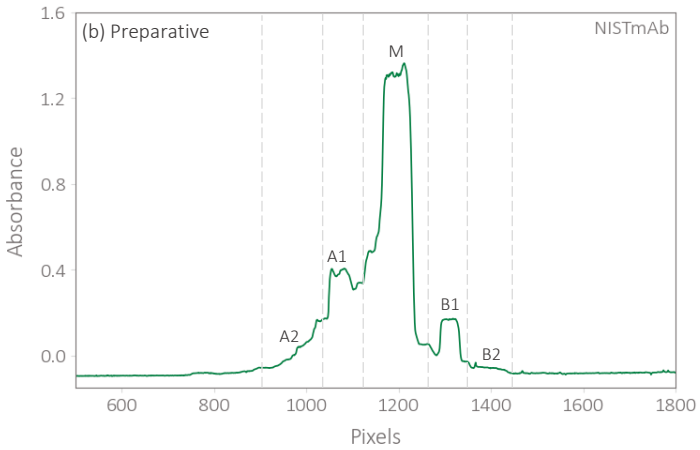
Contact: info@aeslifesicences.com for more parts.

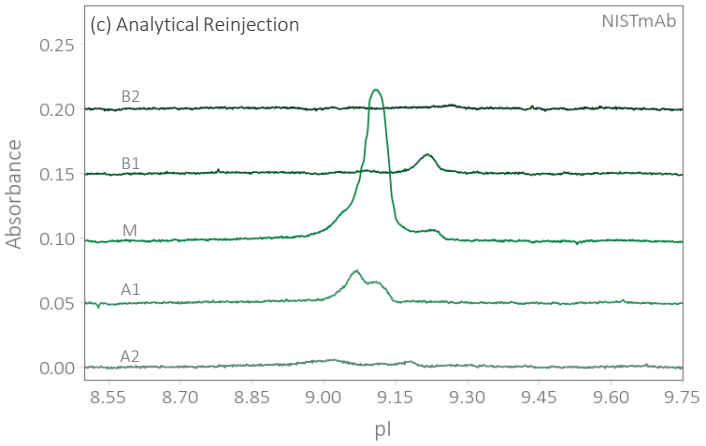
icIEF Fractionation

Overview of Capabilities for icIEF Fractionation on CEInfinite

Throughput	Versatility	Dedicated AES Consumables
✓ Reproducibility Pressure mobilization and durable capillary coatings.	✓ Dynamic Fractionation Custom selection via software for each fraction interval.	✓ CEInfinite Coatings Optimize mobilization with AD, MC, and FC coatings.
✓ Automation Pool fractions over consecutive unattended runs.	✓ Method Development 45 min/run to rapidly optimize fraction purity.	✓ Prep Cartridge Lifetime 50+ runs for most samples with no upper limit.
✓ High Throughput Collect ~100 µg of sample per day.	✓ Reverse Polarity Elute acidic fractions first for improved purity.	✓ AESlytes® Narrow-range AESlytes® to optimize fraction purity (p7).







(d)	Initial Area	Est. Fraction Purity	Est. Fraction Recovery	Est. Amount per 25 runs
B2	1.43%	87.4%	65.4%	2.4 µg
B1	8.50%	89.2%	63.5%	13.5 µg
M	58.86%	86.3%	87.3%	129.2 µg
A1	16.97%	46.5%	58.4%	25.0 µg
A2	14.24%	63.0%	61.0%	21.8 µg

Fig 5. (a) The NISTmAb icIEF profile using the analytical capillary cartridge (100 µm diameter). (b) The NISTmAb icIEF profile using the preparative capillary cartridge (320 µm diameter). This is before pressure mobilization, with the different regions selected for fractionation. (c) The icIEF profile of the collected fractions reinjected into the analytical cartridge. (d) An estimation of the fraction purity, recovery, and quantity calculated based on the a starting concentration of 2.5 mg/mL in a 4.02 µL capillary. The recovery estimation assumes no sample loss during transfer to the collection vials.

icIEF-MS Coupling

→ Resolution and Reliability. The pressure mobilization and transfer capillary, along with a carefully designed icIEF-MS interface, have enabled robust performance.

→ Critical Reagents. AES has leveraged its expertise in chemical synthesis to develop carrier ampholytes and permanent MC coatings for better electrospray ionization.

→ MS Instrument Compatibility. The platform works with various MS brands, including Thermo Fisher, Waters, and Agilent. That said, it performs best when used with the Thermo Scientific Q Exactive Plus and a nanospray emitter.

→ Seamless Method Transfer. Switch between analytical icIEF, fractionation, and icIEF-MS with limited changes to the cartridge and method. As a result, this instrument provides a highly flexible icIEF strategy.

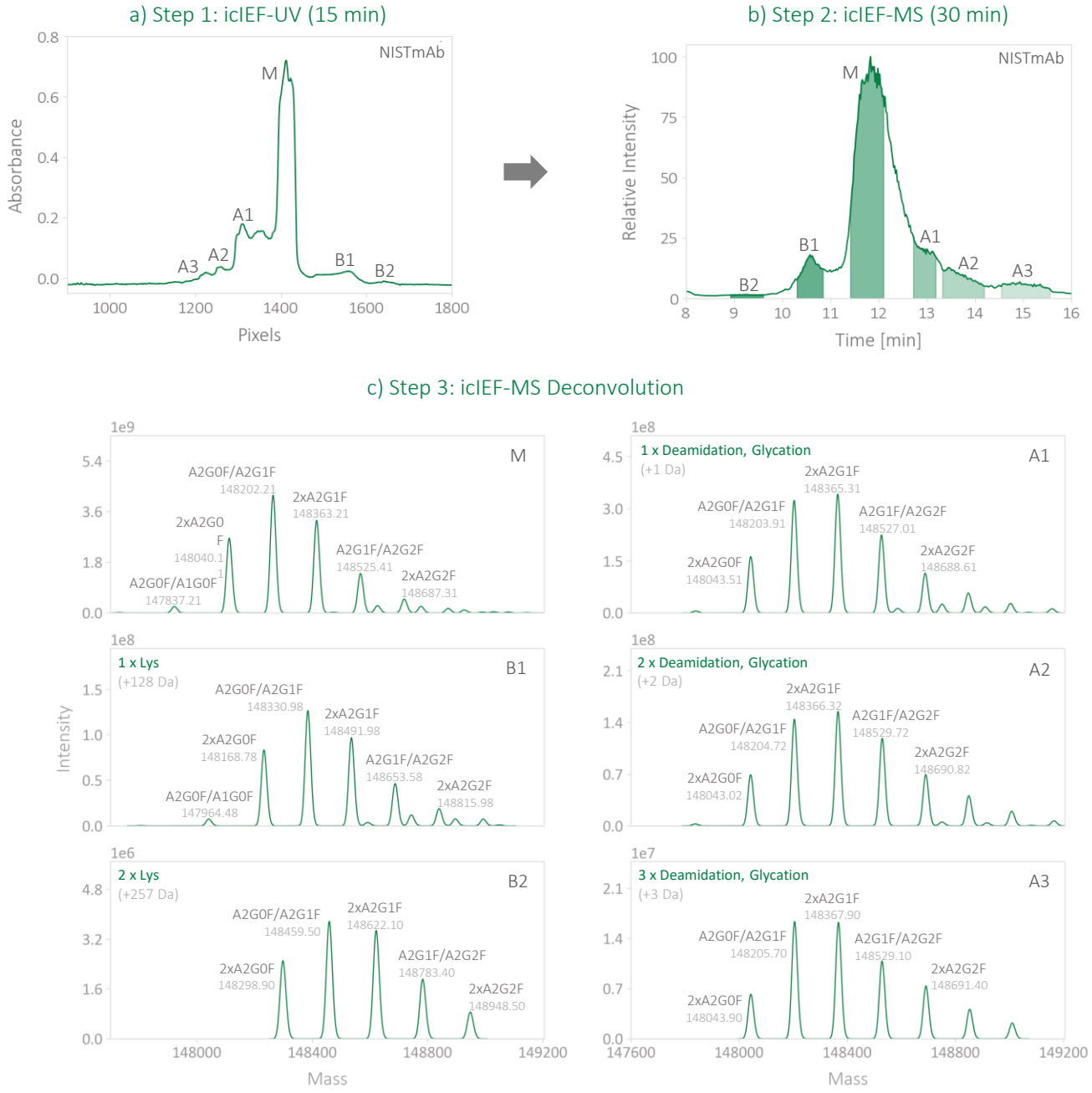


Fig 6. a) An icIEF profile before mobilization and direct MS coupling. b) Total ion electropherogram (TIE) from MS with the corresponding icIEF peaks. c) The deconvolution of each icIEF region which highlights a variety of critical quality attributes (CQAs) including charge heterogeneity, glycosylation, deamidation, and C-terminal lysines.

Multifunction icIEF Preparative model

Part# CE30003

- icIEF fractionation and icIEF-MS online.
- Fraction peaks < 0.1 pI, purity up to 99%.
- Reverse polarity for enhanced acidic species fraction.
- High yield fractionation up to 1mg per day*.
- Chromeleon™ control icIEF-MS, advanced capabilities with Nano ESI or Native MS.
- Life-time support.

*Depending on sample.

Preparative icIEF Consumables and Kits

Cartridges

Part#	Coating	Info
CP00207P	FC-Fluorocarbon Hydrophobic	Auto fractionation
CP00307P	AD-Acrylamide derivative Hydrophilic	Auto fractionation
CP00303M	AD-Acrylamide derivative Hydrophilic	icIEF-MS online
CP00507P	MC-Methylcellulose Very hydrophilic	Auto fractionation
CP00503M	MC-Methylcellulose Very hydrophilic	icIEF-MS online

Kits

Part#	Info
101002	Condition Solution, 5 mL
100052	icIEF/CEInfinite Fractionation System Test Kit,
101004	10mM Arginine Solution
101010	Electrolyte Solutions kit (Anolyte, Catholyte), 100 mLx2.
100052	icIEF/CEInfinite Fractionation System Test Kit, 30 mL.
SV00801	PP, 300uL Sample Vial, Snap Ring, 100/pack with pre-cut caps.

Contact : info@aeslifesicences.com for more parts.

icIEF Services

AES has built a state-of-the-art icIEF facility and developed a team of experienced scientists and engineers (Fig 7).

The Industry Challenges. The next generation of icIEF is upon us, and many are looking to access the most advanced tools. This is especially true as the industry places more of a premium on in-depth information, and becomes more ambitious in the development of therapeutics with complex charge profiles.

Maximizing the potential of these technologies can require a lot of time and resources, and everyone has a unique set of timelines when deciding to commit to such an investment.

The Advanced Electrophoresis Solutions. AES services are helping companies adapt to the next generation of icIEF, regardless of their current timelines. For example, this is a great option for high-priority projects that simply cannot wait. Alternatively, we can assist you with smaller projects as you look to become more familiar with our technology.

→ Extensive Experience. Our leadership has been heavily involved in the development of icIEF for several decades. Furthermore, we have a team of capable scientists and engineers who have been working through some of the industry's most difficult icIEF problems to date.

→ Method Development. We routinely support customers developing icIEF methods for complex samples. For example, we can recommend different carrier ampholytes, capillary coatings, and system parameters.

→ High-Throughput Fractionation. Several CEInfinite units are available at our facility, allowing us to rapidly collect more than 50 µg of each icIEF peak. We can then run buffer exchange on the fractions before freezing and returning them for further analysis.

→ icIEF-MS and Peptide Mapping. Our facility includes a Thermo Scientific Q Exactive Plus mass spectrometer to run icIEF-MS with excellent resolution and sensitivity (Fig 8). We also have a Thermo Scientific Vanquish UHPLC system for in-depth peptide mapping after fractionation.

→ Custom Carrier Ampholytes. Thanks to our unique understanding of their design and synthesis, we can help alleviate many of the concerns associated with carrier ampholytes: a) custom formulations for optimal stability and resolution, b) profile matching of existing icIEF curves, c) guaranteed lot-to-lot consistency, d) resupply services, and e) regulatory filing support.

→ Instrument Evaluation. We are happy to accept samples from those who want to test the CEInfinite.

Part#	Item	Delivery Time	Sample Requirements	Standard Delivery Results
300000	Service-Critical Reagent	Depending on customer request.		
300001	Service-Analytical	1-2 weeks	>500 µL; >1 mg/mL	For mAb, Bispecific, Trispecific, ADC, Enzyme, AAV, Fusion proteins and other complex molecules. Report: UV Spectra; Reproducibility of pIs and peak areas.
300002	Service-Fractionation	4-8 weeks	Depending on customer request	Get three groups (Acidic, Main, Basic) 50 µg for each group; >60% purity. Fractions collections resend to customer. Report: including UV spectra and reinjection purity spectra.
300003	Service-icIEF-MS	2-6 weeks	>500 µL; >10 mg/ mL	Report: UV Spectra; MS TIC spectra; corresponding deconvolution result;
300004	Service-Peptide Mapping	>8 weeks	>500 µL; >10 mg/ mL	Report: UV Spectra; MS TIC spectra; corresponding deconvolution result; MS mapping results.

Table 1. list of icIEF charge variant services offered by AES.

CEInfinite icIEF Fractionation System Overview

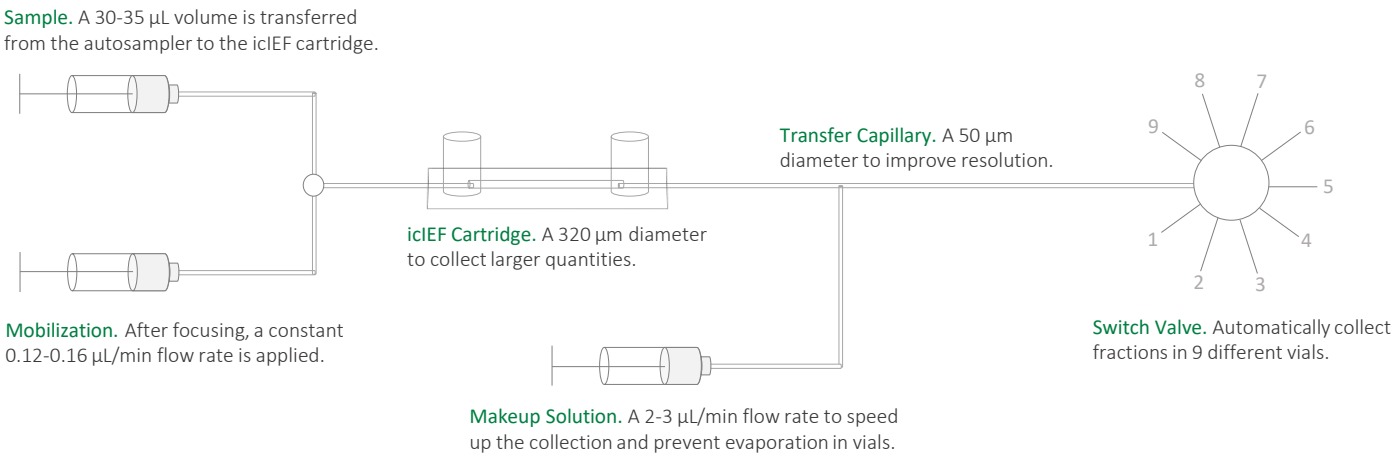


Fig 9. (a) An overview of the CEInfinite fractionation system. The sample is loaded in the autosampler before being transferred to the icIEF cartridge. The capillary diameter is increased from 100 µm to 320 µm to increase the amount of sample collected. The separation takes place for 10-20 minutes before the mobilization is activated. At this point, the charge variant peaks move toward the transfer capillary while the specific regions are defined for collection. The software calculates the time it takes for each region to reach the switch valve, which then adjusts automatically to direct the fractions into their respective vials. (b) The icIEF-MS system is a similar setup with the following exceptions. The icIEF cartridge has a 200 µm diameter. The make-up solution includes acetonitrile and formic acid for better electrospray ionization efficiency. Finally, the switch valve is replaced with direct coupling to a mass spectrometer.

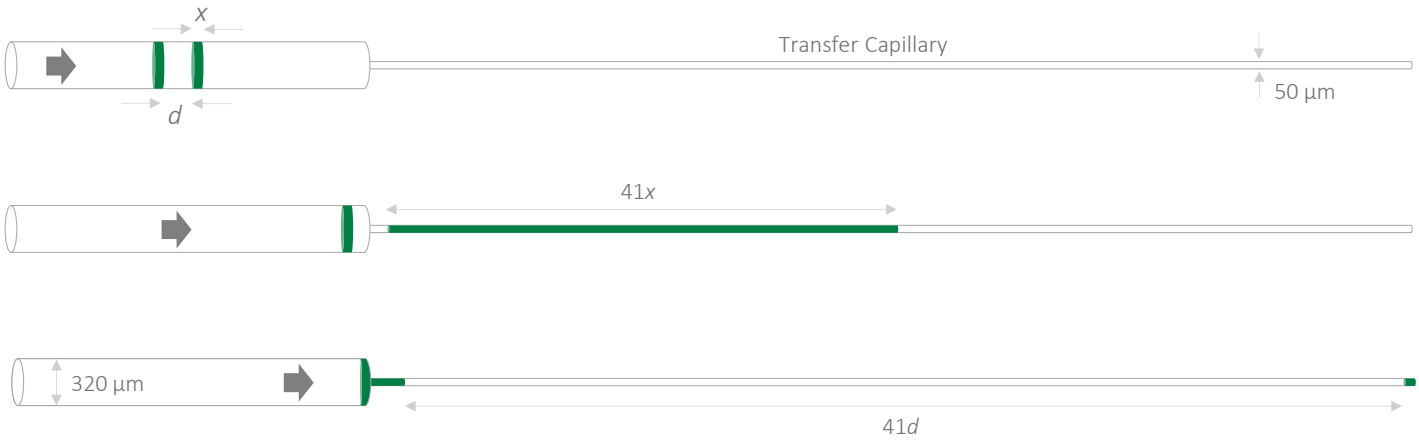


Fig 10. AES' IDTT (Inner Diameter Transfer Technology) patent. As the charge variant zones go from the 320 µm diameter icIEF capillary to the 50 µm diameter transfer capillary, the separation distance will increase by approximately 41 times, and therefore reduce the cross-contamination caused by molecular diffusion and the parabolic velocity flow profile. The pressure mobilization enables excellent consistency and speed while avoiding the chemical mobilization additives that can cause instabilities related to Joule heating and pH gradient disruption. The last peaks to elute will have the worst purity due to the extra-column effects influencing the preceding samples. Fortunately, pressure mobilization also supports reversed polarity to allow the acidic variants to elute first for a higher purity.

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Supporting Information

19. Agilent Application Note 2017.
C. Wenz. Comparison of Different Brands of Carrier Ampholytes for Monoclonal Antibody Charge Heterogeneity Analysis by Capillary Isoelectric Focusing.

20. Electrophoresis 2022.
S. Marden. Global intercompany assessment of ICIEF platform comparability for the characterization of therapeutic proteins. 43, 1050-1058.

21. US Pharmacopeia Technical Note 2023.
Charge Variant Analysis of USP Monoclonal Antibody Reference Standards.

22. US Pharmacopeia at CE Pharm 2022.
N. Peckham. Development and Characterization of Reference Standards to Support Analysis of Charge Variants.

Specifications

CEInfinite

Detection Mode	280nm absorbance
pI Range	2.5 – 11.5
Sensitivity (LOD)	3 ug/mL (Based on mAb)
Detection Dynamic Range	2 logs
High Voltage Range	0 – 8000 Volts (3 stages of focusing)
Sample Throughput	Up to 12 injections per hour
Minimum sample volume	15 uL
pI resolution	0.03 pI (3-10 ampholyte)
pI CV	<1%
CV for Peaks≥ 10% composition	≤4%
Linearity	>0.995
Fractionation amount	Up to 10 µg isomer/run, 25-35 runs per 24 hours. (molecule-dependent)
Operation Mode	Automatic (Manual mode available)
Detector Noise	Less than 0.001 AU, 280 nm
Weight	30 pounds (14 kg)
Height x Width x Depth	54 cm x 33 cm x 30 cm

Auto-Sampler

Model	840
Sample Capacity	84+3 vial tray, 96 well plate
Sample Tray Temperature	4 - 40°C
Typical Sample Volume	15 µL
Electrical Requirement	95 – 240 VAC, 50 – 60 Hz
Height x Width x Depth	36 cm x 30 cm x 57.5 cm
Weight	46 pounds (21 kg)
Working Temperature	10 - 40°C
Humidity	20 – 80% RH

Injection Pump

Syringe Size	250 µL/5mL
Electrical Requirement	100 – 240 VAC, 50 – 60 Hz
Height x Width x Depth	12.14 cm x 10.8 cm x 24.1 cm
Weight	2.72 pounds (1.23kg)
Working Temperature	5 – 40°C

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