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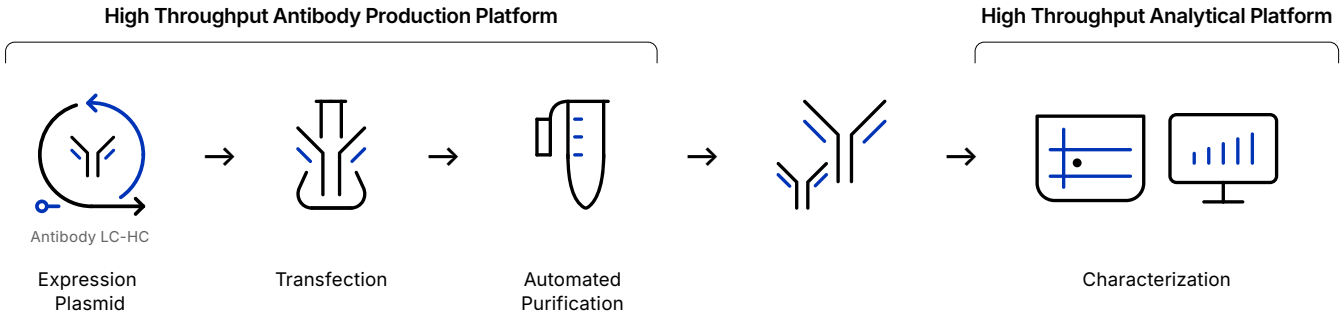
Instruments

Integrating Upstream Production with Downstream Rapid Characterization for *High-Throughput Antibody Development*

Monoclonal antibodies were produced on a high-throughput production platform and were rapidly profiled by Turbo CE-SDS and icIEF on board mixing (OBM), revealing set-wise purity, fragmentation and charge trends that support fast, informed candidate triage.

High-throughput (HTP) production of monoclonal antibodies (mAbs) is a necessary step for accelerating the screening and clone selection process, but equally critical is the availability of analytical tools that can quickly but reliably provide results that guide early selection and other informed decisions. This application note describes a streamlined workflow that combines an HTP recombinant antibody production platform with capillary electrophoresis methods on the Maurice™ System. Over 100 recombinant antibodies were produced on a HTP platform, purified, and then analyzed for charge heterogeneity with imaged capillary isoelectric focusing (icIEF) and capillary electrophoresis-sodium dodecyl sulfate (CE-SDS). Both icIEF and CE-SDS resolved distinct peaks in all antibodies, signifying important structural properties that could potentially influence the selection process.



Materials and Methods

All materials used in this study are listed in Table 1.

TABLE 1

Material	Vendor	Catalog No.
Wild-type HEK293 Cells	-	-
PEI 25K™ Transfection Reagent	Kyfora Bio	23966
Corning® Mini Bioreactors	Millipore Sigma	CLS431720
AmMag™ Protein A Magnetic Beads	Genscript	L00695
AmMag SA PLUS System	Genscript	L01013
PBS (pH 7.4)	Thermo Fisher Scientific	10010031
Zeba™ Spin Desalting Columns		89892
NanoDrop™ 2000 Spectrophotometer		ND-2000
Maurice OBM System	R&D Systems	**090-158**
Maurice cIEF Cartridge		**PS-MC02-C**
Maurice cIEF Method Development Kit*		**PS-MDK01-C**
Maurice Turbo CE-SDS Application Kit*		**PS-MAK01-TS**
Maurice Turbo CE-SDS Cartridge		**PS-MC02-TS**
Iminodiacetic Acid (IDA)	Sigma-Aldrich	56781
NDSB195		480001

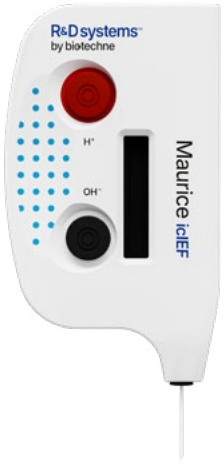
Table 1. Materials and reagents used in this study.
*The Maurice cIEF Method Development and Turbo CE-SDS Application Kits contain all the necessary reagents for icIEF and CE-SDS analysis on Maurice, respectively.

High Throughput Antibody Expression and Purification

Wild-type HEK293 cells were transfected with a proprietary expression plasmid containing the antibody heavy and light chain sequences of interest using 2 mg/mL PEI 25K™ Transfection Reagent in 4 mL of transfection media. After incubating for 3 hours at 100 rpm, transfectants were transferred to the Corning® mini bioreactors along with 20 mL of production media. The bioreactors were incubated at 37°C with 5% CO2 for 6-7 days, shaking at 250 rpm. At the end of the production period, conditioned media was clarified at 1500 rpm for 5 min and transferred to new tubes. Conditioned media containing purified antibodies was then incubated overnight with 100 uL AmMag™ Protein A Magnetic Beads with end-over-end rotation, followed by purification on the AmMag™ SA Plus System. The beads were washed once with PBS + 0.05% Tween-20, twice with PBS, and eluted once for 6 minutes and immediately neutralized with 120 µL 2 M Tris (pH 8.5). Antibodies were then buffer exchanged into PBS (pH 7.4) using Zeba™ Spin Desalting Columns and antibody titer was determined using a NanoDrop™ 2000 Spectrophotometer.

Analysis of Antibodies with Maurice Supersonic icIEF and Turbo CE-SDS

icIEF



The Maurice System's On-Board Mixing (OBM) feature was used for icIEF analysis, where samples are mixed with reagents automatically in the system right before analysis. 25 uL of antibody samples at 0.5 mg/mL in PBS (5X concentration) were placed in a Maurice 96-well plate. Ampholyte mix vials contained 1.25X Master Mix, which was made of a final master mix containing 0.35% MC, (0.5% each) 4% Pharmalytes (3-10), 4M Urea, IDA 10 mM, NDSB 10 mM, 10% SimpleSol, and pI Markers (0.5% each) 4.05 and 9.50. With OBM, the instrument brings the final sample concentration to 1X, mixing 4-parts 1.25X Master Mix and 1-part 5X sample.

The samples were loaded onto a Maurice instrument along with the Maurice cIEF cartridge, with running conditions set at focusing for 0.5 min at 250 V, 0.5 min at 500 V, 0.2 min at 1000 V, 0.2 min 2000V, 0.2 min at 3000 V, then 7 min at 4300 V. Data were acquired with native fluorescence (NF) detection, and all data were analyzed using Compass for iCE Software v4.4.

CE-SDS

Sample buffer mixture was prepared by adding 50 µL Internal Standard, 120 µL of either 250 mM IAM (non-reduced analysis) or 14.2 M β-ME (reduced analysis), 68.5 µL 10% SDS, 1.2 mL 1X CE-SDS PLUS Sample Buffer. Samples were diluted to a final concentration of 0.5 mg/mL with 1X PBS. The sample buffer mixture and 0.5 mg/mL samples were then mixed at 1:1 (10 µL each), to a final concentration of 0.25 mg/mL. Samples were heated at 70°C for 10 minutes, cooled on ice for 5 minutes, then 80 µL deionized water was added for improved stacking and centrifuged for 5 min. Samples were loaded onto a Maurice 96-well plate and placed into the Maurice instrument and injected for 8 seconds at 3500 V and separated at 4200 V for either 8 minutes (non-reduced) or 5.5 minutes (reduced). The data were analyzed with Compass for iCE software.

Results

Owing to the large number of antibodies expressed and analyzed, the data shown are for three select groups.

Set 1

Figure 1A shows stacked profiles of a set of eight heat-stable antibodies, analyzed with CE-SDS under non-reduced conditions. Dominant intact species are observed for all eight samples, with expected variations between them as they are different antibodies. Additionally, all samples showed minor levels of fragmentation, as quantified in Table 2. Analysis of the same set of antibodies under reduced conditions resulted in well-resolved heavy chain (HC) and light chain (LC) peaks, along with minor peaks that are likely non-glycosylated heavy chain peaks (NG HC). Minor differences in relative migration times are observed between samples (Figure 1B).

FIGURE 1A

Set 1 - Non-Reduced CE-SDS

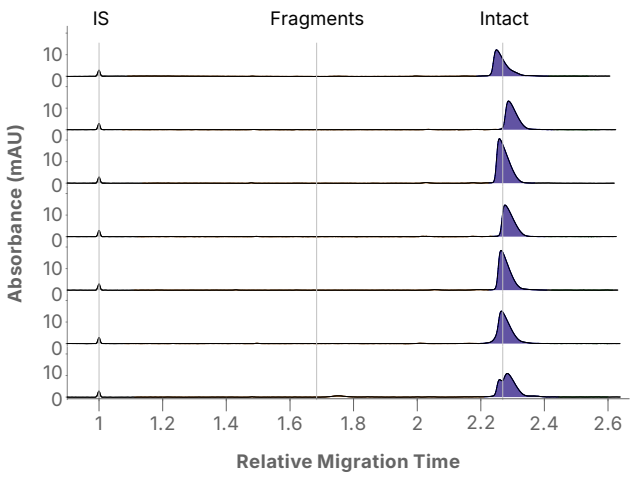


FIGURE 1B

Set 1 - Reduced CE-SDS

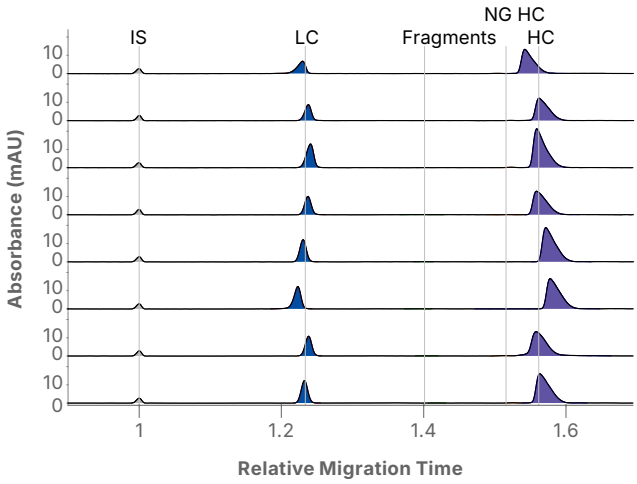


Figure 1. CE-SDS profiles for Set 1. A. Non-reduced separations show dominant intact species with minor fragmentation across eight antibodies. B. Reduced separations resolving HC, LC, and minor NG HC peaks with small migration differences between samples.

TABLE 2

Set 1 - Percent Peak Area (n=8)

	Non-Reduced		Reduced		
Sample	**Intact**	**Fragments**	**HC**	**NG HC**	**LC**
1	92.8	5.9	68.3	0.2	31.4
2	94.3	5.4	67.6	0.5	31.9
3	97.4	2.6	68.0	0.5	31.5
4	95.9	3.5	67.1	0.2	32.7
5	96.6	3.0	69.9	0.0	30.1
6	96.4	3.3	65.5	0.0	34.5
7	96.3	3.1	68.8	0.7	30.5
8	91.1	8.2	67.0	0.3	32.6

Table 2. Set 1, CE SDS percent peak areas under non reduced (Intact, Fragments) and reduced (HC, NG HC, LC) conditions.

Set 1 *(continued)*

Figures 2A and 2B show one representative electropherogram of the charge profile of one sample with icIEF analysis and stacked charge profiles of the entire set of eight samples. As listed in Table 3, the acidic and main species are abundant, with minor basic peaks. Different pI values between samples are observed.

FIGURE 2A

FIGURE 2B

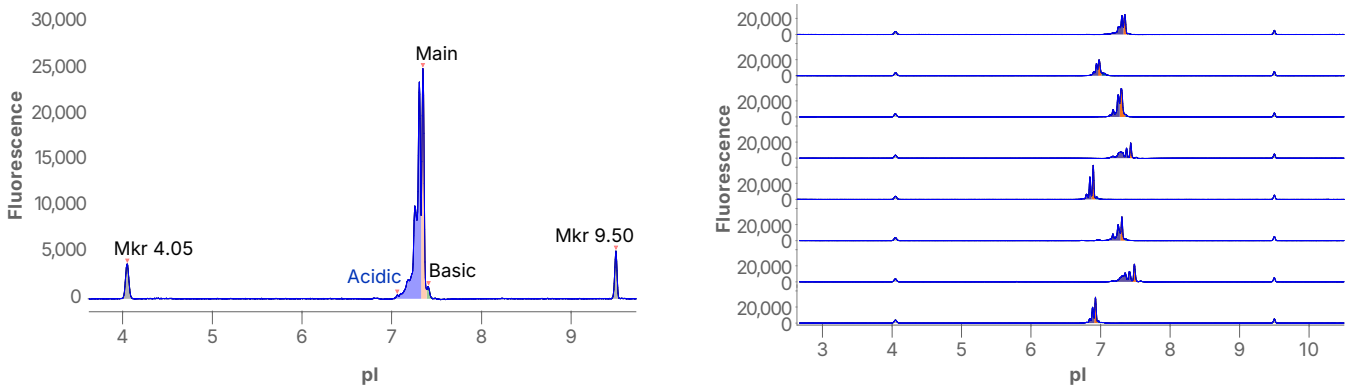


Figure 2. icIEF charge profiles for Set 1. A. Representative electropherogram of one antibody highlighting acidic, main, and basic species. B. Stacked icIEF profiles for all eight antibodies showing set-wise charge heterogeneity and differing pI values.

TABLE 3

Set 1 - Percent Peak Area (n=8)

Sample	Acidic	Main	Basic
1	62.2	35.6	2.2
2	39.6	44.8	15.6
3	48.0	48.2	3.8
4	70.3	28.0	1.7
5	44.6	49.3	6.1
5	59.7	36.3	4.0
7	68.4	29.7	1.9
8	44.5	48.4	7.1

Table 3. Set 1 icIEF percent peak areas for acidic, main, and basic species across eight antibodies.

Set 2

The next set of antibodies analyzed was found to be heat-labile, which is evidenced by the high number of fragments detected with CE-SDS under non-reduced conditions (Figure 3A). Under reduced conditions, the expected LC and HC peaks were detected for all samples (Figure 3B). Quantitative data for CE-SDS analysis are presented in Table 4.

FIGURE 3A

Set 2 - Non-Reduced CE-SDS

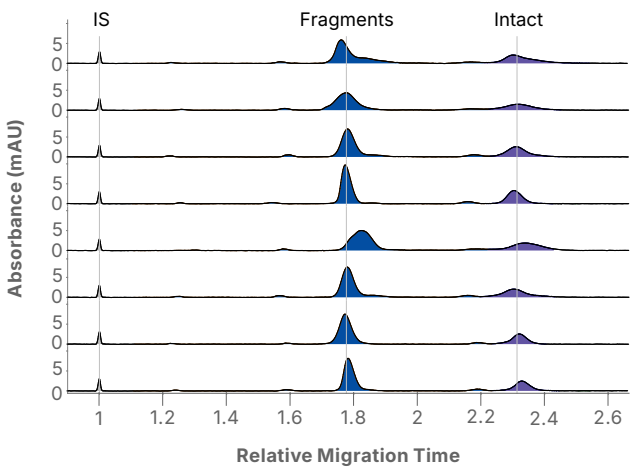


FIGURE 3B

Set 2 - Reduced CE-SDS

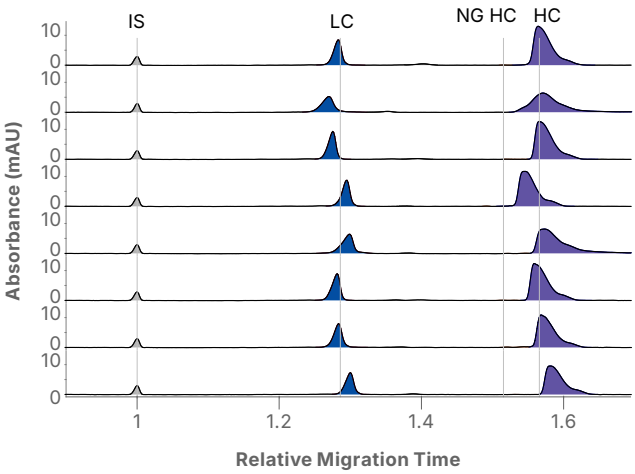


Figure 3. CE-SDS profiles for Set 2. A. Non-reduced runs with high fragment content indicating heat sensitivity. B. Reduced runs resolving expected HC and LC peaks across samples.

TABLE 4

Set 2 - Percent Peak Area (n=8)

	Non-Reduced		Reduced		
Sample	**Intact**	**Fragments**	**HC**	**NG HC**	**LC**
1	26.7	73.3	72.4	0.1	27.6
2	23.3	76.7	68.8	0.0	31.2
3	25.8	74.2	70.4	0.1	29.5
4	26.3	73.7	70.4	0.1	29.5
5	26.5	73.5	69.2	0.1	30.7
6	25.3	74.7	69.8	0.2	30.0
7	22.5	77.5	67.9	1.1	31.0
8	23.6	76.3	68.8	0.1	31.1

Table 4. Set 2 CE SDS percent peak areas under non reduced and reduced conditions; high fragment levels reflect heat-related degradation.

Set 2 *(continued)*

When analyzed with icIEF, this set of heat-labile samples was characterized by an abundance of acidic species, as seen in Figures 4A and 4B. This increase in acidic peaks is likely attributed to sample degradation spurred by exposure to heat. The percentage peak areas for observed peaks are listed in Table 5.

FIGURE 4A

Set 2 - icIEF

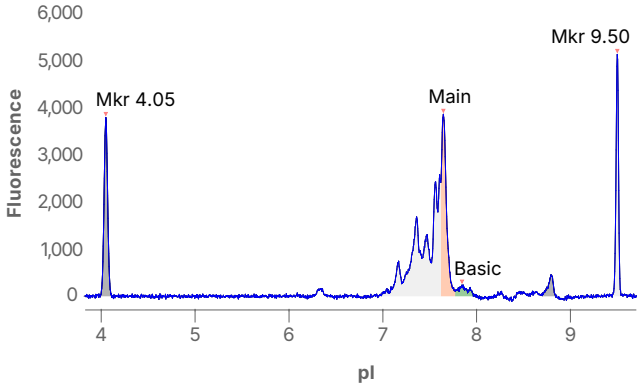


FIGURE 4B

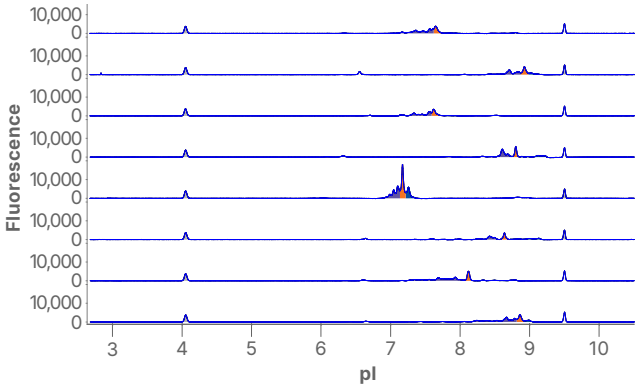


Figure 4. icIEF charge profiles for Set 2. A. Representative electropherogram enriched in acidic species. B. Stacked profiles for all eight antibodies showing a shift toward acidic variants.

TABLE 5

Set 2 - Percent Peak Area (n=8)

Sample	Acidic	Main	Basic
1	66.0	30.4	3.6
2	52.9	34.3	12.8
3	59.3	37.5	3.2
4	59.3	27.6	18.5
5	37.1	42.1	20.8
5	47.7	26.8	25.6
7	64.3	24.8	10.9
8	66.9	24.4	8.7

Table 5. Set 2 icIEF percent peak areas for acidic, main, and basic species; enrichment in acidic variants compared with other sets.

Set 3

Similar to Set 1, Set 3 was heat-stable, with the samples displaying mainly intact peaks with modest fragments when analyzed with CE-SDS under non-reduced conditions (Figure 5A). Under reduced conditions, the broad HC peaks generated suggest conformational or reduction-related heterogeneity commonly observed in mAb heavy chains following purification, rather than true fragmentation (Figure 5B). Quantitative data for CE-SDS analysis are shown in Table 6.

FIGURE 5A

Set 3 - Non-Reduced CE-SDS

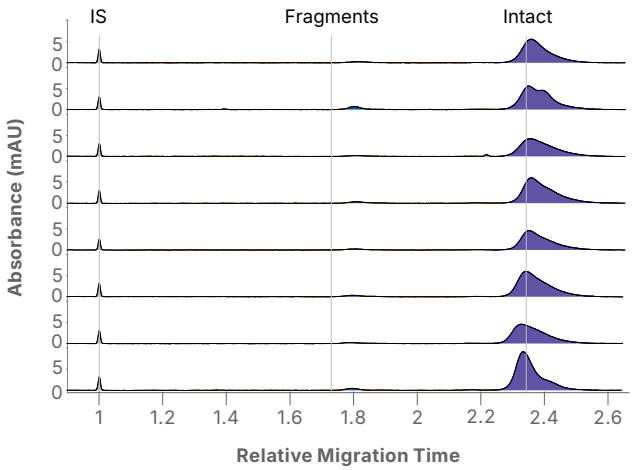


FIGURE 5B

Set 3 - Reduced CE-SDS

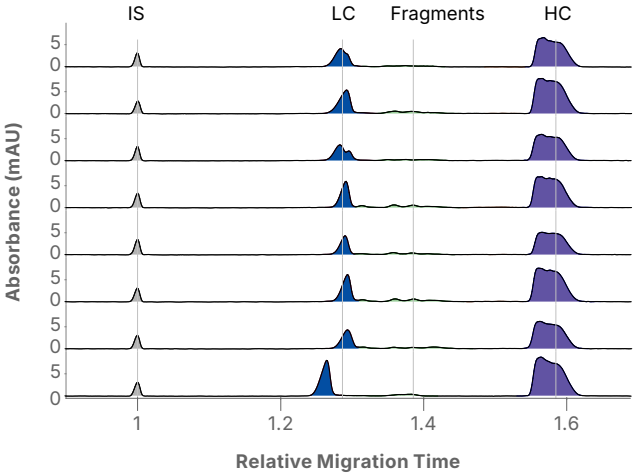


Figure 5. CE-SDS profiles for Set 3. A. Non-reduced separations showing mainly intact peaks with modest fragmentation. B. Reduced separations with broad HC peaks suggestive of conformational/reduction-related heterogeneity rather than true fragmentation.

TABLE 6

Set 3 - Percent Peak Area (n=8)

	Non-Reduced		Reduced			
Sample	**Intact**	**Fragments**	**HC**	**NG HC**	**LC**	**Fragments**
1	92.8	7.2	70.8	0.0	26.9	2.4
2	88.6	11.4	69.6	0.3	24.1	6.1
3	92.0	8.0	70.1	0	25.5	4.5
4	95.1	4.9	69.1	0.8	22.1	8.0
5	94.1	5.9	69.4	0.5	22.3	7.8
6	91.5	8.5	69.0	0.5	22.7	7.8
7	94.9	5.1	69.9	0	22.4	7.7
8	93.3	6.7	70.0	0	27.9	2.1

Table 6. Set 3 CE SDS percent peak areas under non reduced and reduced conditions, including Fragments (R) measured under reducing conditions.

Set 3 *(continued)*

icIEF analysis of this final set of antibodies revealed higher amounts of basic species compared to the other two sets. Figure 6A is a representative electropherogram of one antibody from this set, while Figure 6B shows stacked profiles of all eight antibodies. Table 7 lists the percentage peak area, where comparatively higher values of the basic species are shown.

FIGURE 6A

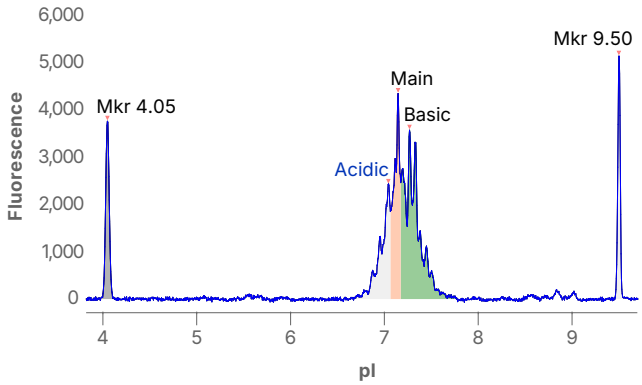


FIGURE 6B

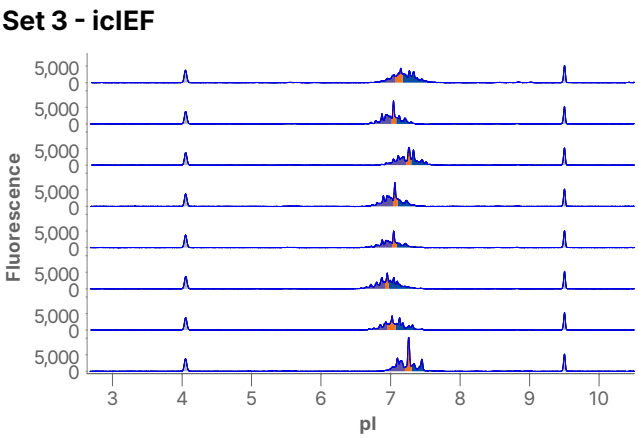


Figure 6. icIEF charge profiles for Set 3. A. Representative electropherogram highlighting comparatively higher basic species. B. Stacked profiles across the set demonstrating a shift toward basic variants relative to Sets 1–2.

TABLE 7

Set 3 - Percent Peak Area (n=8)

Sample	Acidic	Main	Basic
1	22.4	25.9	51.7
2	45.2	25.9	29.0
3	42.2	22.3	35.5
4	47.8	24.5	27.7
5	48.9	23.8	27.3
5	37.9	17.0	45.1
7	27.2	28.5	44.3
8	39.6	33.4	27.1

Table 7. Set 3 icIEF percent peak areas showing comparatively higher basic species across the set.

Conclusion

Recombinant antibodies are essential research tools and are extensively applied in both diagnostics and therapeutics. In biotherapeutic development, challenges that arise with large scale screening campaigns include production of recombinant antibodies at small-scale in sufficient throughput and robust analytical methods to identify candidates with optimal manufacturability. This study demonstrates the production of ~100 recombinant antibodies in one run and their subsequent analysis with icIEF and CE-SDS on the Maurice instrument. Turbo CE-SDS quickly checks size and purity, while icIEF with on-board mixing automates sample prep to keep samples intact. The latter also maps charge variants—acidic, main, and basic—and tracks pI shifts that signal potential post-translational modifications or degradation. Together, these methods deliver fast and highly reproducible readouts that inform clone selection, highlight heat lability versus stability, and reduce bottlenecks for downstream development. In this study, CE-SDS separated heat-labile from heat-stable groups, while icIEF tracked the related acidic and basic shifts within each set. This high-throughput antibody production platform, paired with Maurice CE-SDS and icIEF, creates a fit-for-purpose toolkit for processing large mAb sets. This combination enables earlier selection of robust candidates and accelerates downstream process and formulation decisions.

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