R&D systems by biotechne

Instruments

An End-to-End Workflow for Rapid CD28 Antibody Development and Multi-Attribute Characterization

Highlights

- **Techniques:** icIEF, CE-SDS, ELISA
- **Platforms:** TcBuster, Ambr 250, Maurice, Ella
- **Therapeutic Modality:** mAb (CD28)
- **Cell Line:** CHO-K1
- CD28 mAb-producing CHO-K1 cells were produced via TcBuster™ transposon-mediated integration.
- The top-producing clone was cultured in an Ambr® 250 high throughput bioreactor under varying conditions; the focus in this study was on two different media and two different temperatures.
- icIEF and CE-SDS assays on the Maurice™ system were used to analyze the mAb charge heterogeneity, purity, and size in these cell supernatants.
- The Ella™ System, built on Simple Plex™ technology, was used to analyze host cell protein (HCP) in these cell supernatants.

Why this workflow matters:

TcBuster™-mediated cell line engineering enables the rapid establishment of stable, high-expressing CHO cell lines and addresses the challenges of clone generation and heterogeneity which often occur with random integration. An Ambr® 250 system allows parallel evaluation of multiple parameters such as media and temperature in a highly controlled scale-down model of production-scale bioreactors, thus solving the bottleneck of evaluating multiple culture conditions at scale. Finally, CE-based analytical characterization using the Maurice™ system and automated HCP analysis on the Ella™ System overcome traditional analytical throughput and sample volume limitations brought about by processes like IEF gels, SDS-PAGE, and ELISAs. Together, these tools provide a robust workflow for quickly generating and analyzing therapeutic mAbs of interest.

Step 1: Electroporate cells with anti-CD28 transposon with TcBuster-M transposase

Step 2: Selection and expansion of stable pools of edited cells

Step 3: Analysis of stable pools for antibody secretion and copy number

Step 4: Single cell cloning from stable pools

Step 5: Analysis of clones for antibody secretion and copy number

Step 6: Screen culture conditions on Ambr 250 for manufacturing potential

Step 7: icIEF and CE-SDS analysis with the Maurice system, HCP analysis with Simple Plex assays

Case Study: Effects of Bioreactor Media and Temperature on Antibody Charge and Size Profiles

Appendix 1 provides details on the materials and methods used in this study.

After TcBuster-mediated gene transfer and development of single cell clones, the top producing clone was cultured in Ambr 250 high throughput bioreactors under four different conditions, in duplicate for 14 days, as shown in Table 1. Cell supernatants were assessed for total IgG by ELISA on days 7 and 14.

TABLE 1

Condition #	Media	Temperature (°C)	IgG g/L (Day 7)	IgG g/L (Day 14)
1	Dynamis	36.5	2.38	5.16
2	Dynamis	33	1.19	5.24
3	ActiPro	36.5	1.61	4.66
4	ActiPro	33	1.48	12.00

Table 1. An outline of different conditions and total IgG ELISA values determined for cell culture in the Ambr 250 bioreactors. ELISA data are the average of two bioreactors.

Results

FIGURE 1

CE-SDS & icIEF Analysis of Cell Supernatants with the Maurice System Show the Size & Charge Profiles of the Monoclonal Antibody

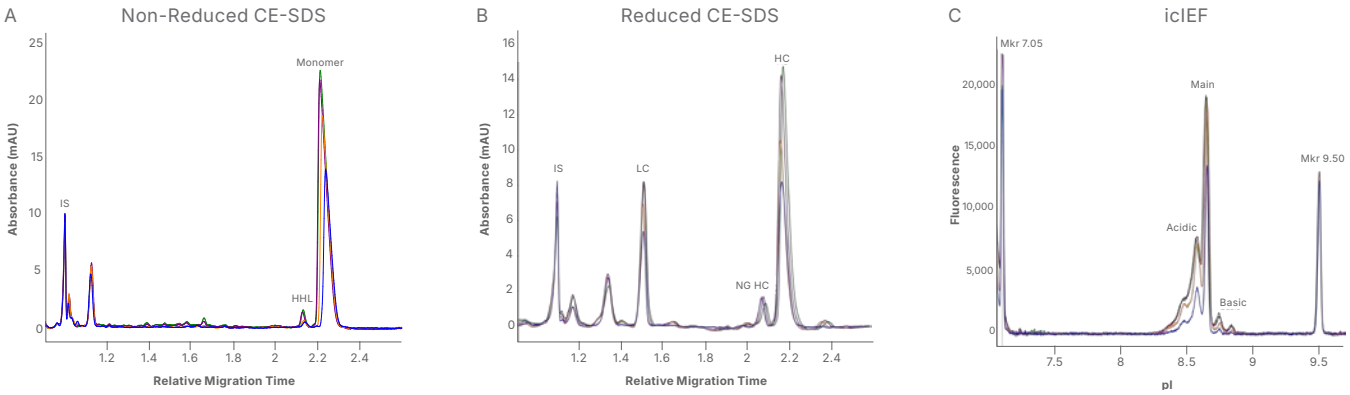


Figure 1. Representative electropherograms of the monoclonal antibody detected in cell supernatants across multiple reactors. CE-SDS under non-reduced conditions (1A) reveals the monomer, with a small percentage of the heavy-heavy-light (HHL) fragment, and under reduced conditions (1B) resolves into the expected heavy chain (HC), light chain (LC), and non-glycosylated heavy chain (NG HC), along with a few other fragments. icIEF analysis (1C) shows peaks resolved into acidic, main, and basic regions.

TABLE 2

Non-Reduced CE-SDS Shows Predominantly Intact Antibody with Modest Increases in High–Molecular-Weight Species Over Culture Duration

% Peak Area (Non-Reduced CE-SDS)					
Media	**Temperature (°C)**	**Monomer**		**HHL**	
		Day 7	**Day 14**	**Day 7**	**Day 14**
Dynamis	36.5	96.5	96	3.0	3.6
Dynamis	33	97.4	96.5	2.0	3.2
ActiPro	36.5	90.2	92.2	2.0	3.9
ActiPro	33	97.8	92.9	**2.1**	**6.2**

Table 2. Non-reduced CE-SDS analysis of CD28 antibody size variants across two different media, two different temperatures, and a culture duration of 14 days. Percent peak area (% PA) values are shown for cultures grown in Dynamis or ActiPro media at either 36.5°C or 33°C. Non-reduced CE-SDS was used to assess intact antibody (monomer) and high–molecular-weight species (HHL) in culture supernatants collected over a span of 14 days, with results shown for Day 7 and Day 14. Across all conditions, the monomer was the predominant species, indicating high mAb integrity throughout the culture period. Modest increases in HHL species (highlighted in bold) were observed at later culture days, particularly with ActiPro at 33°C. These results demonstrate that culture conditions can influence the accumulation of size variants over time while maintaining a consistent proportion of intact antibody.

TABLE 3

Reduced CE-SDS Confirms Consistent Heavy and Light Chain Composition Across Media and Temperature Conditions

% Peak Area (Reduced CE-SDS)							
Media	**Temperature (°C)**	**HC**		**NG HC**		**LC**	
		Day 7	**Day 14**	**Day 7**	**Day 14**	**Day 7**	**Day 14**
Dynamis	36.5	65.9	65.9	1.9	1.5	32.2	32.6
Dynamis	33	66.2	66.2	2.0	1.7	31.8	32.1
ActiPro	36.5	59.4	61.7	8.1	4.7	32.5	33.6
ActiPro	33	67.2	64.7	1.0	2.3	31.8	33.0

Table 3. Reduced CE-SDS analysis of CD28 antibody size variants across different culture conditions. Reduced CE-SDS analysis was performed to evaluate heavy chain (HC), light chain (LC), and non-glycosylated heavy chain (NG HC) species in supernatants harvested over 14 days, with Dynamis or ActiPro media at either 36.5 or 33°C. % PA values are shown for each condition at Day 7 and Day 14 of culture, where HC and LC distributions were consistent, and NG HC species remained minor in comparison. The stability of reduced CE-SDS profiles may indicate preserved antibody integrity and proper assembly across media, temperature, and culture duration.

TABLE 4

icIEF Reveals Media- and Temperature-Dependent Shifts in Antibody Charge Variant Distribution Over Time

		% Peak Area (icIEF)					
Media	**Temperature (°C)**	**Acidic**		**Main**		**Basic**	
		Day 7	**Day 14**	**Day 7**	**Day 14**	**Day 7**	**Day 14**
Dynamis	36.5	42.4	43.7	55.3	50.0	2.3	6.3
Dynamis	33	41.1	43.7	50.4	46.1	8.5	10.2
ActiPro	36.5	35.8	49.1	56.1	44.3	8.1	6.6
ActiPro	33	33.7	43.6	58.3	50.0	8.0	6.4

Table 4. icIEF characterization of charge variants of CD28 as a function of media, temperature, and time. icIEF analysis was used to characterize antibody charge heterogeneity by resolving acidic, main, and basic species in culture supernatants collected at Day 7 and Day 14. % PA values are shown for cultures grown in Dynamis or ActiPro media at 36.5 °C or 33 °C. While the main peak remained the most abundant across all conditions, there were notable shifts in the amount of acidic and basic species observed, depending on the condition. While further experiments are required to determine the exact cause of these shifts, these results indicate that base media composition and/or culture temperature do influence mAb charge heterogeneity.

TABLE 5

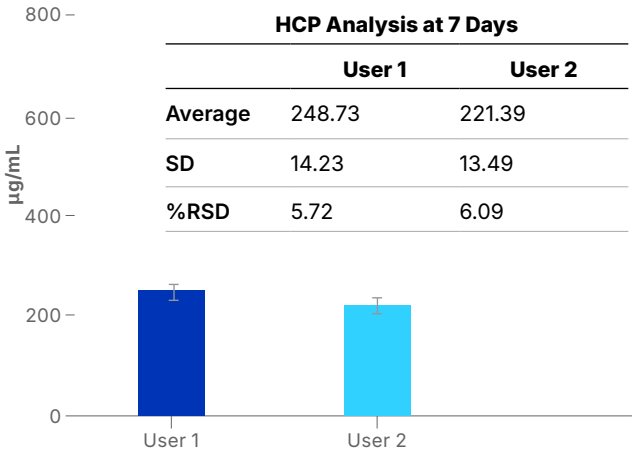
Simple Plex Assay Shows Time-Dependent Changes in Host Cell Protein Levels During Culture

# Days of Culture	µg/mL
7 Days	308.5
10 Days	511.8
12 Days	536.4
14 Days	496.3

Table 5. Host cell protein concentration in culture supernatants measured over time with the Ella™ system. Host cell protein (HCP) levels in culture supernatants were quantified using the Ella Simple Plex platform at multiple culture time points. Samples collected at Days 7, 10, 12, and 14 were analyzed from a representative Ambr 250 bioreactor, and results are reported as HCP concentration (µg/mL). HCP levels increased during the culture period, reaching a maximum at a later time point before stabilizing, consistent with the expected accumulation of host cell–derived impurities during extended culture.

FIGURE 2

Simple Plex Assay of Host Cell Protein



HCP Analysis at 7 Days

	User 1	User 2
Average	248.73	221.39
SD	14.23	13.49
%RSD	5.72	6.09

Figure 2. Reproducibility of host cell protein measurements with the Ella system. Host cell protein levels at Day 7 were measured using the Ella Simple Plex platform by two independent users to assess assay reproducibility. Bar plots show average HCP concentration (µg/mL) for each user, with error bars representing standard deviation. The embedded table summarizes mean values, standard deviation (SD), and percent relative standard deviation (%RSD). Consistent HCP measurements between users demonstrate the reproducibility and robustness of automated Simple Plex assays for upstream impurity monitoring using minimal sample volume.

Conclusion

- TcBuster transposon-mediated integration enabled rapid generation of a stable, high-producing CHO-K1 cell line expressing a CD28 monoclonal antibody, supporting accelerated cell line development workflows.
- Use of the Ambr 250 high-throughput bioreactor system enabled efficient evaluation of media and temperature effects on antibody productivity and quality attributes using minimal material.
- Non-reduced and reduced CE-SDS analysis with the Maurice™ system confirmed that intact antibody and proper heavy and light chain composition were maintained across culture conditions, with modest, condition-dependent changes observed in size variant profiles over time.
- icIEF analysis with the Maurice system revealed media- and temperature-dependent changes in antibody charge variant distribution during culture, highlighting the sensitivity of charge heterogeneity to upstream process parameters.
- Automated host cell protein analysis using the Ella™ platform demonstrated time-dependent changes in HCP levels during culture, while also confirming high assay reproducibility with minimal hands-on time and sample volume.

Together, these results demonstrate how integrated upstream process development and multi-attribute characterization can be performed rapidly and reproducibly to inform cell culture optimization and product quality assessment. To learn more about TcBuster, Maurice, Ella platforms, and more integrated solutions, visit **rndsystems.com**.

Appendix 1: Materials, Methods, and Supplemental Data

APPENDIX 1A

Materials and Methods

Material	Vendor	Catalog #
TcBuster-M Transposase mRNA	R&D Systems	TCB-001.1-100
TcBuster Compatible DNA Plasmid (encoding CD28 antibody and puromycin resistance)	In-House	N/A
HyClone™ ActiPro™ Cell Culture Media	Cytiva	SH31039.03
HyClone CDM4MAb Cell Culture Media		SH30802
HyClone Cell Boost™ 7a Supplement		SH31119.01
HyClone Cell Boost 7b Supplement		SH31120.02
InstiGRO™ CHO Plus	Advanced Instruments®	NC1668724
AntiClumping Agent	Gibco	0010057 AE
CD CHO Medium		10743029
Dynamis™ Medium		A2661501
EfficientFeed™ B+ 3X Supplement		A3937501
Puromycin Dihydrochloride	SigmaAldrich®	P9620
Neon™ Electroporation System	Thermo Fisher	MPS100
Neon Transfection System 100 µL Kit		MPK10025
Maurice 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 A1: Materials and Methods used in this study.

Culture and Bioreactor Conditions

Two media formulations were used throughout generation of clones and stability studies. Growth medium, composed of CD CHO medium supplemented with 6 mM GlutaMAX and 0.5% AntiClumping Agent, was used for development of stable pools, cloning, and initial clone screening. Throughout cell line generation, CHO-K1 cells were cultured at 37°C and 5% CO_2 on an orbital shaker kept between 90-120 rpm.

The Ambr 250 bioreactor experiment included two media formulations: Dynamis medium supplemented with Gibco Efficient Feed B+ and glucose or ActiPro medium supplemented with CellBoost 7a and 7b. All fed batch reactor vessels were inoculated at 0.5 x 10^6 cells/mL with temperature set to 36.5°C. Supplements were administered by the Ambr throughout the 14-day run

according to manufacturer specifications for each culture medium. Antifoam was added as needed, and pH was set to 7.0 and controlled throughout the production run. Four bioreactor conditions were tested in duplicate for each clone: each media formulation kept at 36.5°C throughout the entire run, and each media formulation with the temperature shifted to 33°C on day 5.

CE-SDS

Samples were prepared by combining 30 µL of reactor supernatant with 50% SDS 1X sample buffer and 4% internal standard. For non-reduced analysis, samples were treated with 5% (v/v) 250 mM iodoacetamide (IAM). For reduced analysis, samples were treated with 5% (v/v) 14.2 M β-mercaptoethanol (BME).

All samples were heated at 70 °C for 10 min, cooled on ice for 5 min, and centrifuged for 5 min. The resulting supernatant was loaded onto the Maurice system along with the Turbo CE-SDS™ cartridge. Non-reduced samples were injected at 3500 V for 8 s and separated at 4200 V for 8 min. Reduced samples were injected at 3500 V for 8 s and separated at 4200 V for 5.5 min.

icIEF

Samples were prepared by combining 2 µL of reactor supernatant with 8 µL of H_2O and 90 µL of master mix. The master mix consisted of 4% ampholytes containing BR1 (broad range pH 3–10) and NR3 (narrow range pH 8–10.5), 0.35% methyl cellulose, 30% formamide, 5% iminodiacetic acid (IDA), 5% nondetergent sulfobetaine (NDSB), 10% SimpleSol™, and pI markers at 0.5% each (pI 7.05, PN 046032; pI 9.5, PN 046035). The samples were loaded onto the Maurice instrument and the running conditions were set at 0.5 min at 500 V, 0.5 min at 1000 V, 0.5 min at 1500 V, 0.5 min at 2000 V, followed by 9 min at 3000 V.

Simple Plex Assay of Host Cell Protein (HCP) :

Product description: Simple Plex™ Assay is for the detection of Human Embryonic Kidney Host Cell Protein (HEK 293 HCP 3G) in Bioprocess samples in Sample Diluent SD19.

Simple Plex™ Assay is for the detection of Human Embryonic Kidney Host Cell Protein (HEK 293 HCP 3G) in Bioprocess samples in Sample Diluent SD19. This assay uses Cygnus Technologies HEK 293 HCP 3G resupply 1 antibodies.

SD19 Concentrate (Diluted 1:10)

Sample Preparation

An appropriate dilution factor for each process matrix should be determined experimentally by assessment of sample linearity and spike recovery. Samples above the ULOQ require further dilution. The cartridge is prepared and run as shown in the protocol referenced **here**.

Reactor samples were diluted 20K-40K in 1X SD19 to create the linear range.

APPENDIX 1B

Supplemental CE-SDS and icIEF Analysis at Intermediate Culture Time Points (Days 10 and 12)

% Peak Area (Non-Reduced CE-SDS)

Media	Temperature (°C)	Monomer		HHL	
		Day 10	Day 12	Day 10	Day 12
Dynamis	36.5	97.6	96.3	2.1	3.4
Dynamis	33	97.7	96.3	2.0	3.4
ActiPro	36.5	89.9	91.6	3.6	3.5
ActiPro	33	93.9	91.9	5.7	7.1

Table B1. Non-reduced CESDS was used to assess intact antibody (monomer) and high–molecularweight species (HHL) in culture supernatants collected at Days 10 and 12. Percent peak area (%PA) values are shown for cultures grown in Dynamis or ActiPro media at 36.5 °C or 33 °C. These intermediate timepoint data are consistent with trends observed at Day 7 and Day 14 in the main Results section.

APPENDIX 2B

% Peak Area (Reduced CE-SDS)

Media	Temperature (°C)	HC		NG HC		LC	
		Day 10	Day 12	Day 10	Day 12	Day 10	Day 12
Dynamis	36.5	66.4	67.0	1.7	1.6	31.8	31.4
Dynamis	33	65.6	65.9	1.5	1.7	32.9	32.5
ActiPro	36.5	59.5	61.1	6.6	5.4	33.9	33.5
ActiPro	33	65.5	64.6	2.4	2.4	32.1	33.0

Table B2. Reduced CE-SDS analysis was performed to evaluate heavy chain (HC), light chain (LC), and nonglycosylated heavy chain (NG HC) species in culture supernatants collected at intermediate culture time points. %PA values at Days 10 and 12 are shown for each media and temperature condition. Consistent HC and LC distributions across conditions further support the stability of antibody chain composition throughout the culture period.

APPENDIX 3B

% Peak Area (icIEF)

Media	Temperature (°C)	Acidic		Main		Basic	
		Day 10	Day 12	Day 10	Day 12	Day 10	Day 12
Dynamis	36.5	47.0	46.9	49.5	47.3	3.6	5.8
Dynamis	33	45.6	40.5	45.3	49.0	9.2	10.5
ActiPro	36.5	39.8	43.8	50.6	48.0	9.6	8.2
ActiPro	33	35.1	37.1	56.2	54.0	8.7	9.0

Table B3. icIEF analysis was used to characterize antibody charge heterogeneity by resolving acidic, main, and basic species in culture supernatants collected at Days 10 and 12. %PA values are shown for cultures grown in Dynamis or ActiPro media at 36.5 °C or 33 °C, and these results are consistent with those shown for Day 7 and Day 14.