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Expansify™ Technical Note- NISTCHO cNISTmAb Production and Quality Assessment

Based on internally validated culture conditions and representative experimental data generated using Expansify™ plates.

Purpose

This culture guide provides representative conditions for monoclonal antibody production using the NISTCHO cNISTmAb producer cell line in the Expansify™ 1-Well Plate, with performance compared against a conventional 500 mL shake flask. The study includes comparative assessment of antibody production yield and product quality, including purity and aggregation, structural integrity, impurity profile, glycosylation, and binding kinetics to the RSV target antigen. Clarified, cell-free culture supernatants from each culture format were provided to Cambridge Antibody Labs for Protein A affinity purification and subsequent characterization of the purified cNISTmAb samples relative to the pre-characterized NIST reference material RM 8671. These analyses were used to evaluate whether culture format influenced antibody production and product quality. Further optimization may be required based on the intended application and desired outcome.

Materials

Materials, reagents, and cultureware used in this study are described in **Expansify™ Cell Culture Methods – NISTCHO (cNISTmAb Producer Suspension CHO Cell Line)**. Refer to the culture methods document for product specifications, recommended consumables, and culture medium.

Experimental Procedure

NISTCHO cNISTmAb producer cultures were established, maintained, monitored, sampled, and harvested according to **Expansify™ Cell Culture Methods – NISTCHO (cNISTmAb Producer Suspension CHO Cell Line)**, including standard seeding, culture maintenance, sampling, and harvest conditions described therein.

For the study described here, the Expansify™ 1-Well Plate and 500 mL shake flask were each operated at a 100 mL working volume and seeded at 3×10^5 viable cells/mL. Cultures were maintained until viability approached the predefined harvest criterion of approximately 80%. The shake flask culture was harvested on Day 8 at 74% viability, while the Expansify™ culture was harvested on Day 9 at 84% viability. Following harvest, culture supernatants were clarified, collected, and stored at −80°C until subsequent cNISTmAb quality assessment by Cambridge Antibody Labs.

Performance Summary

Production & Yield

The Expansify™ 1-Well Plate supported cNISTmAb production comparable to the 500 mL shake flask under matched culture conditions. Recovered protein concentration and total protein yield were similar between formats, with no increase in endotoxin detected in either sample above threshold. Under the conditions evaluated, these results demonstrate that static production in the Expansify™ 1-Well Plate achieved antibody recovery comparable to the shake flask control.

Culture Format	Protein Concentration (mg/mL)	Recovered Volume (mL)	Total Recovered Protein (mg)	Endotoxin by LAL
500 mL shake flask	0.90	94	84.6	<0.05 EU/mL
Expansify™ 1-Well Plate	1.04	88	91.52	<0.05 EU/mL

Purified antibody samples were maintained in Tris-acetate buffer at neutral pH and stored at −80°C until use.

Purity and Aggregate Profile

Both the shake flask- and Expansify™-produced cNISTmAb samples demonstrated high purity across reducing and non-reducing SDS-PAGE, analytical size-exclusion chromatography (SEC), and CE-SDS analyses. The Expansify™-produced sample exhibited a slightly lower proportion of high-molecular-weight species than the shake flask-produced comparator, indicating a modestly lower aggregate-associated fraction under the conditions evaluated.

Thermal Stability

Differential scanning fluorimetry showed similar thermal transition profiles for cNISTmAb produced in the shake flask and Expansify™ formats. The two culture-derived antibody samples exhibited comparable thermal stability, with measured melting temperatures approximately 1–1.5°C lower than those of the purified NIST reference antibody.

Process-Related Impurities

CHO host cell protein levels were comparable between the shake flask- and Expansify™-produced cNISTmAb samples. Residual host cell DNA was lower in the Expansify™-produced sample, with approximately one-third the level detected in the shake flask-produced comparator.

Under the conditions evaluated, these results indicate that antibody production in the Expansify™ 1-Well Plate did not increase the measured process-related impurity burden and was associated with lower residual host cell DNA in the analyzed sample.

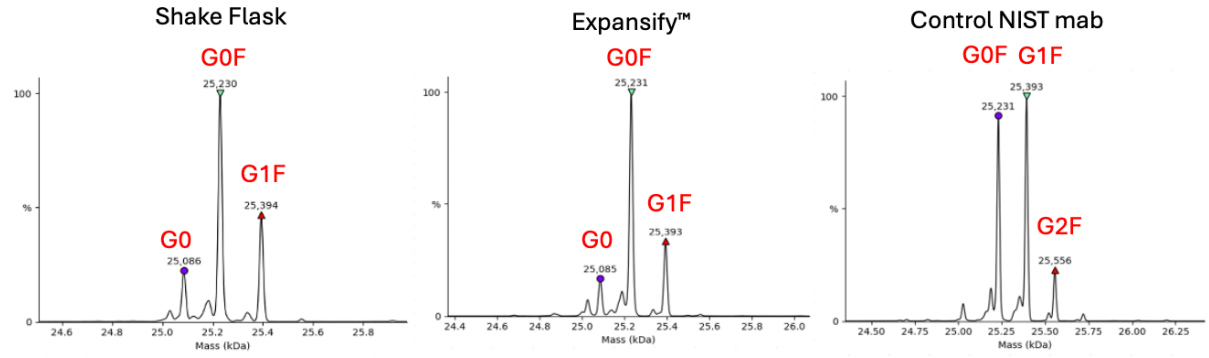
Glycan Profile

The purified NIST reference antibody served as the benchmark for glycan profile comparison. The shake flask- and Expansify™-produced cNISTmAb samples were broadly comparable to the NIST reference, although differences were observed in the relative abundance of specific glycoforms, including G0F. Importantly, the Expansify™- and shake flask-produced antibodies exhibited highly similar glycoform distributions, indicating that cNISTmAb produced in the Expansify™ 1-Well Plate maintained a glycosylation profile closely aligned with the conventional shake flask production format under the conditions evaluated.

Researchers at Cambridge Antibody Labs noted that the differences observed between the experimentally produced cNISTmAb samples and the NIST reference material may reflect differences in upstream production conditions, including culture medium composition and host cell background. Both the Expansify™ and shake flask samples were produced using the NISTCHO cell line under matched culture conditions, whereas the NIST RM 8671 reference material was originally produced in a murine suspension cell culture system. Therefore, differences relative to the reference material may not be attributable to culture format alone.

Figure 1. Comparative Glycoform Profiles of cNISTmAb Samples

Mass-based glycoform profiles of cNISTmAb produced in the Expansify™ 1-Well Plate and shake flask compared with the purified NIST reference antibody.



RSV Antigen-Binding Kinetics and Affinity

The purified NIST reference antibody served as the benchmark for RSV antigen-binding performance. The Expansify™- and shake flask-produced cNISTmAb samples demonstrated strong target binding, with association rate constants within the same order of magnitude as the NIST reference. Dissociation rate constants were below 1.0×10^{-5} s^{-1} for all three samples, with corresponding K_D values in the low-picomolar range or below.

Under the conditions evaluated, cNISTmAb produced in the Expansify™ 1-Well Plate retained RSV antigen-binding kinetics and affinity comparable to the shake flask-produced antibody and purified NIST reference.

Sample	Ka (1/Ms)	Kd (1/s)	KD (M)
NIST mab	1.21E^{06}	<1.0E^{-05}	<8.3E^{-12}
Shake flask	3.28E^{06}	<1.0E^{-05}	<3.0E^{-12}
Expansify™	1.60E^{06}	<1.0E^{-05}	<6.3E^{-12}

Overall Performance Summary

Under the conditions evaluated, the Expansify™ 1-Well Plate demonstrated overall cNISTmAb production performance comparable to the conventional shake flask, while showing favorable differences in selected product-quality attributes. Antibody yield, purity, thermal stability, glycoform profile, and RSV antigen-binding performance were broadly aligned between formats, while the Expansify™-produced material exhibited lower high-molecular-weight species and lower residual host cell DNA.

Collectively, these results demonstrate that static production in the Expansify™ 1-Well Plate can achieve antibody output and key quality attributes comparable to, and in selected measures favorable to, conventional shake flask culture.