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NISTCHO Cell Culture and Fed-Batch Bioreactor Protocols

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Abstract

The NISTCHO cell system (RM 8675) provides a standardized Chinese hamster ovary (CHO) cell culture platform for studies of recombinant monoclonal antibody production and bioprocess characterization. Reproducible recovery, expansion, cultivation, and monitoring of NISTCHO cells are important for generating materials with controlled and documented process histories. This Special Publication describes an integrated upstream workflow beginning with recovery of cryopreserved NISTCHO cells and continuing through automated cell concentration and viability measurement, preparation of chemically defined feed media, seed-train expansion, and fed-batch cultivation in a single-use stirred-tank bioreactor. The procedures define operating conditions for temperature, agitation, dissolved oxygen, pH, feeding, glucose supplementation, antifoam addition, sampling, harvest, and initial clarification. A representative 14-day process profile is included to illustrate typical NISTCHO culture behavior. The procedures are intended to facilitate reproducible implementation of the NISTCHO upstream process and generation of cNISTmAb-containing harvest material for downstream processing and analytical studies.

Keywords

Bioprocessing, cNISTmAb, Chinese Hamster Ovary Cells, Cell Culture, Fed-batch, Monoclonal Antibody, NISTCHO.

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Executive Summary

The NISTCHO upstream process is a laboratory-scale workflow for recovery, expansion, and fed-batch cultivation of NISTCHO cells for production of cNISTmAb. The workflow begins with controlled thawing of a cryopreserved NISTCHO vial (RM 8675), followed by post-thaw recovery and automated measurement of viable cell density and viability. Cells are expanded through a seed train and used to inoculate a single-use stirred-tank bioreactor at an initial viable cell density of approximately 0.5×10^6 1/mL.

The fed-batch culture is operated for 14 days at 37 °C with dissolved oxygen controlled at 40 %, pH maintained between 6.9 and 7.1, and agitation initiated at 160 rpm. A 2:1 mixture of Advanced CHO Feed 1 and Cellvento 4Feed COMP is added on Days 3, 5, 7, 9, 11, and 13. Glucose is supplemented to a target concentration of 8 g/L, and antifoam is added according to a culture-age-dependent schedule. The culture is sampled throughout the run for cell concentration, viability, cell diameter, pH, glucose, and other process measurements as applicable.

The bioreactor procedure was developed using the Eppendorf BioBLU 3c single-use bioreactor configuration described in this publication. Implementation on other bioreactor platforms may require adaptation of equipment-dependent operating parameters, such as agitation, gas flow, aeration, and control strategies, to account for differences in vessel geometry, mixing, mass transfer, and instrumentation. However, biological and process targets, including inoculation cell density and viability, culture temperature, dissolved oxygen and pH targets, feed timing and composition, glucose target, and culture duration, may serve as reference points when translating the NISTCHO process to other appropriately configured bioreactor systems.

On Day 14, the culture is harvested and clarified by centrifugation. The resulting supernatant is used for cNISTmAb titer determination and subsequent downstream purification. The representative process profile included in this document is provided as an example of expected process behavior and is not a statistically established acceptance specification.

1. Introduction

Chinese hamster ovary (CHO) cells are widely used as mammalian hosts for the production of recombinant therapeutic proteins, including monoclonal antibodies, because they support complex protein folding, assembly, and post-translational modification [1,2]. Advances in cell-line engineering, culture media, feeding strategies, and bioreactor process control have enabled high-density CHO cultures and substantially increased recombinant protein productivity [1-4].

Fed-batch cultivation is a widely used mode for recombinant CHO production because nutrient supplementation can extend culture duration and support high product titers while retaining relatively straightforward process operation [3-5]. Media composition, nutrient delivery, pH, dissolved oxygen, temperature, and other culture conditions can influence cell growth and productivity and can also affect product quality attributes [3-5]. Consequently, well-defined culture and monitoring procedures are important when comparing process performance across experiments and laboratories.

NISTCHO was developed as an industry-relevant recombinant CHO reference cell line (RM 8675) that produces a non-originator version of the NISTmAb IgG1 [6]. The selected NISTCHO clone was characterized for growth, productivity, product quality, and transgene integration, and its robustness was evaluated in an interlaboratory study [6]. NIST subsequently recommended standardized nomenclature for the NISTCHO cell line and its CHO-expressed monoclonal antibody product, cNISTmAb, to promote consistent reporting of materials derived from this reference system [7].

This publication provides an integrated upstream procedure for NISTCHO cell recovery, viable cell counting, feed-media preparation, seed-train expansion, fed-batch bioreactor cultivation, and harvest. The procedure consolidates the laboratory methods used for these operations into a single reproducible workflow and is intended to support transparent implementation and reporting of NISTCHO upstream bioprocess experiments. The bioreactor procedure was developed using the Eppendorf BioBLU 3c single-use bioreactor. Implementation on other bioreactor platforms may require adaptation of equipment-dependent operating parameters to account for differences in vessel geometry, mixing, aeration, mass transfer, and process-control configuration. However, the biological and process targets described in this procedure, including cell

density, cell viability, glucose concentration, and feed timing, may serve as reference targets when adapting the NISTCHO process to other appropriately configured bioreactor systems.

2. Warning & Safety

2.1. Cell Culture, Chemical, and Instrumentation Safety

The minimum personal protective equipment required for laboratory work includes protective eyewear, gloves, a laboratory coat, long pants, and closed-toe shoes. Refer to the applicable Safety Data Sheet (SDS) for each chemical and reagent before use and follow institutional requirements for chemical and biological waste disposal.

Cryopreserved vials and liquid nitrogen present cryogenic hazards. Bioreactor operation may involve pressurized gases, heated components, rotating equipment, electrical equipment, pumps, and sterile fluid-transfer assemblies. Personnel should be trained in the safe operation of the equipment used in this procedure.

2.2. Aseptic Handling

Cell recovery, seed expansion, media transfer, sampling, and other open manipulations should be performed using appropriate aseptic technique in a biological safety cabinet (BSC). Consumables, reagent bottles, and equipment entering the BSC should be disinfected with 70 % isopropanol (IPA) as appropriate.

3. Scope

The objective of this procedure is to provide a reproducible laboratory method for recovery and cultivation of NISTCHO cells through production of clarified culture supernatant containing cNISTmAb. The method includes cryovial thawing, cell counting and viability measurement, feed-media preparation, seed-train expansion, setup and operation of a 2 L working-volume single-use bioreactor, scheduled feeding and glucose supplementation, antifoam addition, process sampling, harvest, initial clarification, and titer measurement.

The conditions in this document describe the current NISTCHO laboratory implementation. The procedure described here was developed over a number of runs and represents the process used in three fully successful operations. Representative data from one of the successful operations are included in Section 11. They should not be assumed to be directly transferable to other CHO cell lines, media systems, bioreactor geometries, or production scales without appropriate evaluation. Nor should it be assumed that the described laboratory implementation is exclusive or the only approach for achieving cNISTmAb production.

4. Process Principle & Measurement Method

The NISTCHO upstream process combines controlled mammalian cell cultivation with routine measurements of culture growth and viability. Cryopreserved cells are recovered into ExCell CD Fusion medium, expanded in shake flasks, and used to prepare an inoculum for a single-use stirred-tank bioreactor. The production culture is operated in fed-batch mode using a defined feed mixture and scheduled additions. Dissolved oxygen, pH, temperature, agitation, gas flow, glucose supplementation, and antifoam addition are controlled or monitored throughout the run.

Viable cell density and viability are measured using a Vi-CELL XR imaging cytometer and trypan blue exclusion. The NISTCHO cell-counting method uses a modified CHO analysis configuration with 100 images per sample. Process samples are collected during the run to document culture behavior and to support comparison among replicate or future experiments.

5. Materials

A list of materials and instrumentation used in the NISTCHO upstream procedure is provided in Table 1.

NOTE: Reagents and equipment are specified by name to more fully describe the process. Other brands may be substituted if the required parameters in the protocol are met and the substitute is demonstrated to be fit for purpose.

Table 1. List of materials and instrumentation for the NISTCHO upstream process.

Item	Vendor / Catalog No.
Isopropanol, 70 %	Fisher / A459-4
ExCell CD Fusion medium	MilliporeSigma / 14366C-1000 mL
NISTCHO cryovial	NIST / RM 8675
ExCell Advanced CHO Feed 1	SAFC / 24368C-1L
Cellvento 4Feed COMP	SAFC / 1.03796.001
Sodium hydroxide	As specified by laboratory method
Hydrochloric acid	Fisher / BF 1758-500
ExCell Advanced CHO Fed-Batch medium	MilliporeSigma
Sodium bicarbonate, 7.5 % (w/v)	Corning / MT25035CI
ExCell Antifoam	MilliporeSigma / 59920C
Glucose solution, 45 %	Cell-culture grade
Vi-CELL XR Concentration Control	Beckman Coulter / 175478
Vi-CELL XR Reagent Pack	Beckman Coulter / 383722
Vi-CELL sample cups	Beckman Coulter / 723908
Vi-CELL XR	Beckman Coulter / 383556
BioBLU 3c Macrosparge single-use vessel	Eppendorf / 1386000300
Biowelder	Sartorius Stedim / 16370
Capacitance probe	ABER FUTURA / 6532-52/PG
Optical dissolved oxygen sensor, 220 mm	Eppendorf / P0720-6660
Optical pH sensor and cable	Eppendorf / P0300-2371
Zero oxygen tablets for InLab sensors	Mettler Toledo / 51300140
Tri-port	Eppendorf
Compression fitting, 12 mm	Eppendorf
Sterilizing-grade filter	≤0.22 µm
Orbital platform incubator	37 °C, 5 % CO2, 135 rpm
Benchtop centrifuge	Suitable for 200 × g and 1000 × g
Water bath	37 °C
Biological safety cabinet	Cell-culture use
Erlenmeyer flasks	125 mL and 1 L, sterile, vented
Conical centrifuge tubes	15 mL, 50 mL, and 250 mL
Serological pipettes	Appropriate sterile sizes
Balance, pH meter, stir plate, and stir bar	Calibrated / fit for purpose

NOTE: The capacitance probe was installed as part of the bioreactor configuration used to develop this procedure; however, capacitance measurements are not required for implementation of the NISTCHO fed-batch protocol described in this protocol. Capacitance-based measurements can provide additional real-time information on viable biomass and culture behavior during CHO cell cultivation [8].

6. Solutions

6.1. Advanced CHO Feed 1 Preparation (without glucose)

1. Fill a beaker to 80% of the final solution volume using Milli-Q water or equivalent cell-culture-grade water. Recommended water temperature is 25 °C to 40 °C.
2. While stirring, slowly add sufficient ExCell Advanced CHO Feed 1 to achieve a final concentration of 34.1 g/L.
3. Continuously stir for 30 min. The product may remain slightly turbid.
4. Raise the pH to 9.5 ± 0.1 using 5 mol/L NaOH. Continue mixing for 10 min; the solution should become clear.
5. Lower the pH to 8.5 using 5 mol/L HCl and continue mixing for 10 min.
6. Bring the preparation to 100 % of the final volume with purified water.
7. Immediately sterile-filter using a low-protein-binding filter membrane.
8. Store at 2 °C to 8 °C protected from light. Discard unused feed after one month.

6.2. Cellvento 4Feed COMP Preparation

9. Slowly add 130.35 g Cellvento 4Feed COMP to 800 mL of room-temperature Milli-Q water or equivalent cell-culture-grade water in a 1 L beaker.

10. Rinse the feed container as necessary to recover residual material and mix for 45 min or until fully dissolved.
11. Slowly add 5 mol/L NaOH to adjust the pH to 7.0 ± 0.3.
12. Add cell-culture-grade water to a final volume of 1 L and confirm the pH (7.0 ± 0.3).
13. Immediately filter using a sterilizing-grade filter with a nominal pore size of ≤0.22 µm.
14. Store at 2 °C to 8 °C protected from light.

6.3. Final Feed Media

Prepare the final fed-batch feed by mixing Advanced CHO Feed 1 and Cellvento 4Feed COMP at a 2:1 volume ratio (Table 2).

Table 2. Final feed-media composition.

Component	Volume	Volume ratio
Advanced CHO Feed 1	600 mL	2
Cellvento 4Feed COMP	300 mL	1
Final feed	900 mL	3

7. Cell Recovery and Counting Procedure

7.1. Preparation of Pre-Warmed Medium

15. Transfer 38 mL of ExCell CD Fusion medium to a 50 mL Eppendorf conical tube labeled “media.”
16. Warm the aliquot in a 37 °C water bath for at least 10 min. Do not maintain the medium at 37 °C for more than 60 min.
17. Retrieve the medium from the water bath, disinfect the exterior with 70 % IPA, wipe, and place in a tube rack inside the BSC.

7.2. NISTCHO Cell Thawing and Recovery

18. Preset a countdown timer for 120 s.
19. Retrieve one NISTCHO cryovial and immediately place it in a 37 °C water bath. Start the timer. Without fully submerging the vial, shake or swirl continuously during thawing and invert end-over-end once every 30 s.
20. After 120 s, remove the cryovial, disinfect the exterior with 70 % IPA, wipe, and place it in the BSC.
21. Using a 2 mL serological pipette, transfer the thawed cells to an empty 15 mL conical tube labeled “cells.”
22. Using a 10 mL serological pipette, slowly add 8 mL of pre-warmed medium to the cell suspension.
23. Centrifuge the cell suspension at 200 × g and 20 °C for 5 min.
24. During centrifugation, transfer 20 mL of pre-warmed medium into a sterile 125 mL Erlenmeyer flask.
25. Return the centrifuged cell suspension to the BSC. Carefully aspirate and discard the supernatant without disturbing the cell pellet.
26. Add the remaining 10 mL of pre-warmed medium to the pellet and resuspend by aspirating and dispensing for two to three cycles.
27. Transfer the resuspended cells to the 125 mL Erlenmeyer flask containing 20 mL of pre-warmed medium.
28. Withdraw three 0.6 mL aliquots for cell counting at the 0 h time point.
29. Incubate at 37 °C, 5 % CO_2, and 135 rpm. Allow the culture to incubate for 24 h before sampling.

7.3. Cell Concentration Measurement

The concentration-control assay is performed using the manufacturer’s concentration-control material (beads Cat No. 175478). A passing result is assigned according to the manufacturer’s specified acceptance criterion for the assigned assay value.

Table 3. Vi-CELL XR concentration control settings.

Parameter	Value
Analysis software version	2.42
Cell type	Conc. Control (50 images)

Parameter	Value
Minimum cell diameter	5 µm
Maximum cell diameter	50 µm
Minimum circularity	0.0
Dilution factor	1.0 (undiluted)
Cell brightness	85 %
Cell sharpness	100
Viable cell spot brightness	75 %
Viable cell spot area	5.0 %
Decluster degree	Low
Aspirate cycles	1
Trypan blue mixing cycles	3

30. Vortex the concentration-control material briefly for approximately 10 s to 12 s.
31. Transfer a 0.6 mL aliquot to a Vi-CELL sample cup and place it in the sample carousel at the assigned position.
32. Log the sample position and sample ID and designate the cell type as the concentration control.
33. Start the measurement queue and verify that the concentration-control result meets the applicable acceptance criterion before analyzing NISTCHO samples.

7.4. NISTCHO Cell Counting

For NISTCHO samples, the manufacturer's CHO analysis settings are modified to increase the number of images captured and analyzed from 50 to 100 per sample.

Table 4. Vi-CELL XR NISTCHO cell-counting settings.

Parameter	Value
Analysis software version	2.42
Cell type	100CHO
Number of images	100
Minimum cell diameter	5 µm
Maximum cell diameter	50 µm
Minimum circularity	0.0
Dilution factor	1.0 (undiluted)
Cell brightness	85 %
Cell sharpness	100
Viable cell spot brightness	75 %
Viable cell spot area	5.0 %
Decluster degree	Medium
Aspirate cycles	1
Trypan blue mixing cycles	3

34. Mix the NISTCHO culture sufficiently to obtain a representative suspension.
35. Transfer the required aliquot to a Vi-CELL sample cup.
36. Enter the sample identification and select the 100CHO cell type.
37. Start the measurement and record viable cell density, total cell concentration, viability, and average cell diameter.

8. Seed Train and Bioreactor System

8.1. Preparation of Seed Train/Inoculum

38. In a 125 mL Erlenmeyer flask, culture a 30 mL working volume (as described in section 7.2, step 29) at 37 °C, 5 % CO_2, and 135 rpm for four days, or until viable cell density reaches approximately 4.3×10^6 1/mL with viability ≥94 %.
39. Remove the flask from the incubator and transfer it to the BSC.
40. Withdraw 0.6 mL of cell suspension for viable cell density and viability measurement.

41. Aseptically transfer 372 mL of ExCell CD Fusion medium to a 1 L Erlenmeyer flask and add 28 mL of cell culture.
42. Incubate the 1 L flask at 37 °C, 5 % CO_2, and 135 rpm for approximately four days, or until viable cell density reaches approximately 4.0 × 10^6 1/mL with viability >94 %.
43. Use this culture as the inoculum for the 2 L working-volume single-use fed batch bioreactor.

8.2. Preparation of Single-Use Fed Batch Bioreactor

NOTE: a BioBLU 3c Macrosparge Bioreactor was used

44. Calibrate the balance and tower peristaltic pumps.
45. Autoclave the capacitance probe, feed-bottle connection tubing, sample manifold, and spare tri-port as applicable.
46. Prepare the feed media as described in Section 6.
47. In the BSC, unpack the bioreactor vessel and close the required clamps.
48. Attach the tri-port to spare port 1 and the capacitance probe to spare port 2.
49. Remove the vessel from the BSC and place it on the scale.
50. Connect the sparge inlet, overlay inlet, and capacitance module.
51. Connect the overhead drive, exhaust heat blanket, and vessel heat blanket.
52. Insert the temperature sensor, DO probe, and optical pH sensor.
53. Tare the vessel mass and turn on air, O_2, and CO_2 supplies.
54. Connect two 1 L bottles of ExCell Advanced CHO Fed-Batch medium.
55. Using a peristaltic pump, transfer approximately 1700 mL of medium into the vessel and record the mass.
56. Set air flow to 0.2 L/min (standard), temperature to 37 °C, agitation to 160 rpm down-flow, and sparge to 100 % air at approximately 0.2 L/min (standard).
57. Allow the vessel to equilibrate overnight.

9. Fed-Batch Cultivation Procedure

9.1. Sensor Calibration and Inoculation

58. Calibrate the DO probe followed by the pH probe. Confirm that the DO control point is 40 %.
59. After DO calibration, enable the three-gas control. Set gas-flow limits to 0.20 minimum and 0.40 maximum as implemented in the laboratory control configuration, and enable overlay. These gas-flow limits are specific to the bioreactor configuration used in this procedure and may require adjustment when implementing the process on other bioreactor platforms to achieve the specified dissolved oxygen and pH control targets. The pH reading should be approximately 6.9.
60. Connect 7.5 % (w/v) sodium bicarbonate to pump 1, ExCell Antifoam to pump 2, 45 % glucose to pump 3, and the inoculum to the designated transfer pump.
61. Add inoculum to achieve an initial viable cell density of approximately 0.5 × 10^6 1/mL (time = 0 days).
62. A representative inoculation uses approximately 300 mL of cell culture with a cell density of 3.2 × 10^6 1/mL added to approximately 1700 mL ExCell Advanced CHO Fed-Batch medium. Record the total volume.
63. Collect a representative sample and measure viable cell density and viability. Initial viability should be >94 %.

9.2. Feed and Glucose Strategy

On Days 3, 5, 7, 9, 11, and 13, supplement culture with Feed and glucose. Specifically, add 100 mL of the final feed prepared in Section 6.3. Before each feed addition, collect the required culture sample for cell measurement and process analytics. Measure glucose concentration using a bioanalyzer and supplement with 45 % glucose solution to a target of 8 g/L.

Table 5. NISTCHO fed-batch feed schedule.

Culture day	Feed addition	Glucose target
3	100 mL	8 g/L
5	100 mL	8 g/L
7	100 mL	8 g/L

Culture day	Feed addition	Glucose target
9	100 mL	8 g/L
11	100 mL	8 g/L
13	100 mL	8 g/L

After feeding, measure offline pH. If the offline value differs by more than ±0.05 pH units from the value displayed by the bioreactor, re-standardize the pH measurement. The bioreactor pH should be maintained between 6.9 and 7.1 during the run.

9.3. Antifoam Strategy

Antifoam is added at 0.5 mL/min for 1 min at frequencies that increase with culture age, as shown in Table 6.

Table 6. Antifoam addition schedule.

Culture period	Addition interval	Addition rate / duration
Day 0-4	1440 min	0.5 mL/min for 1 min
Day 4-7	720 min	0.5 mL/min for 1 min
Day 7-11	360 min	0.5 mL/min for 1 min
Day 11-14	180 min	0.5 mL/min for 1 min

9.4. Sampling and Process Monitoring

Samples should be collected at the defined process time points and identified by run, culture day, and sample time. At minimum, viable cell density, viability, average cell diameter, pH, and glucose should be recorded as applicable. Feed additions, glucose additions, antifoam additions, sensor re-standardizations, and process deviations should also be documented.

10. Harvest and Initial Clarification

64. On Day 14, collect the final culture sample and measure viable cell density and viability.
65. Use the peristaltic pump to transfer the cell culture from the bioreactor.
66. Centrifuge the harvested culture at 1000 × g for 10 min.
67. Repeat the clarification centrifugation a second time.
68. Collect the clarified supernatant in a separate container.
69. Measure cNISTmAb titer.
70. Use the clarified supernatant as starting material for the applicable downstream purification procedure.

11. Representative Process Profile and Reporting

Table 7 provides a representative NISTCHO fed-batch process profile. These values illustrate the behavior of a typical run and are not statistically derived acceptance limits.

Table 7. Representative 14-day NISTCHO fed-batch process profile.

Day	VCD (10^6 1/mL)	Viability (%)	Avg. diameter (µm)	pH
0	0.43	97.9	14.23	6.95
1	0.80	97.7	14.70	7.05
2	1.43	96.4	14.58	7.02
3	2.78	97.1	14.50	6.96
4	4.47	97.5	14.81	6.96
5	8.19	97.9	15.02	6.97
6	12.16	97.4	14.94	7.01
7	15.25	95.8	14.47	7.04
8	20.26	95.3	14.50	7.01
9	21.61	91.4	14.59	7.05
10	20.53	86.7	15.12	7.03
11	21.96	87.9	14.20	7.08
12	19.65	84.2	15.14	7.08

Day	VCD (10^6 1/mL)	Viability (%)	Avg. diameter (µm)	pH
13	18.52	79.6	15.44	7.13
14	17.90	79.7	15.08	6.98

NOTE: Viable cell density, viability, and average cell diameter were measured using the Vi-CELL XR cell viability analyzer. The manufacturer specifies a counting accuracy and counting repeatability of ±10 % for viable cell density and viability. For cell diameter optical resolution is 1 µm per pixel. pH values were recorded from the bioreactor pH measurement system and are reported to the resolution of the recorded measurement (0.01 pH unit).

11.1. Calculations

Viability (%) = (viable cell concentration / total cell concentration) × 100

Initial viable cell density = (inoculum viable cell density × inoculum volume) / final culture volume

For an inoculum of approximately 300 mL with a cell density of 3.2 × 10^6 1/mL and a final culture volume of approximately 2000 mL, the theoretical initial viable cell density is approximately 0.48 × 10^6 1/mL.

11.2. Principal Process Conditions

Table 8. Summary of principal process conditions.

Process parameter	Target / operating condition
Post-thaw incubation	37 °C, 5 % CO_2, 135 rpm
Initial bioreactor VCD	≈0.5 × 10^6 1/mL
Initial bioreactor viability	>94 %
Initial production-medium volume	≈1700 mL
Nominal working volume after inoculation	≈2 L
Culture temperature	37 °C
Initial agitation	160 rpm
Initial air flow	≈0.2 L/min
DO control point	40 %
Culture pH	6.9 to 7.1
Feed days	3, 5, 7, 9, 11, 13
Feed volume	100 mL per scheduled addition
Feed composition	2:1 Advanced CHO Feed 1:Cellvento 4Feed COMP
Glucose target	8 g/L
Culture duration	14 days

12. Summary

This publication provides a consolidated procedural method for NISTCHO cell recovery, cell counting, feed-media preparation, seed-train expansion, 2 L working-volume fed-batch cultivation, and harvest. Additional characterization of the NISTCHO fed-batch process and resulting cNISTmAb, including evaluation of process performance and product attributes, is described in Bolla et al. [8]. The defined operating conditions and measurement settings are intended to support reproducible implementation and transparent reporting of the NISTCHO upstream process. The clarified culture supernatant produced by this procedure contains cNISTmAb and can be used as starting material for downstream purification and analytical characterization.

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Appendix A. List of Symbols, Abbreviations, and Acronyms

Symbol / abbreviation	Definition
BSC	Biological safety cabinet
CHO	Chinese hamster ovary
CO_2	Carbon dioxide
cNISTmAb	Recombinant monoclonal antibody produced by NISTCHO
DO	Dissolved oxygen
HCl	Hydrochloric acid
IPA	Isopropanol
LN2	Liquid nitrogen
NaOH	Sodium hydroxide
PPE	Personal protective equipment
rpm	Revolutions per minute
SDS	Safety Data Sheet
VCD	Viable cell density

Appendix B. Example NISTCHO Run Sequence

Stage / day	Primary activity
Cryovial recovery	Thaw NISTCHO vial; centrifuge and resuspend; establish 30 mL recovery culture
Seed expansion	Expand in 125 mL flask; measure VCD and viability
Inoculum expansion	Expand 28 mL culture into 372 mL ExCell CD Fusion medium in 1 L flask
Day 0	Inoculate bioreactor at ≈0.5 × 10^6 cells/mL; record baseline measurements
Days 1-2	Daily process monitoring
Day 3	Sample; add 100 mL feed; adjust glucose to 8 g/L
Day 5	Sample; add 100 mL feed; adjust glucose to 8 g/L
Day 7	Sample; add 100 mL feed; adjust glucose to 8 g/L
Day 9	Sample; add 100 mL feed; adjust glucose to 8 g/L
Day 11	Sample; add 100 mL feed; adjust glucose to 8 g/L
Day 13	Sample; add 100 mL feed; adjust glucose to 8 g/L
Day 14	Final sample; harvest; centrifugation; collect clarified supernatant; measure titer