NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

Date of Issue:
04 June 2025

Reference Material 8675
NISTCHO, Clonal CHO-K1 Cell Line Producing cNISTmAb
REFERENCE MATERIAL INFORMATION SHEET

Purpose: This Reference Material (RM) is intended to provide a publicly accessible, industry relevant monoclonal antibody expressing cell line for biomanufacturing research and development. This RM can be used for a variety of purposes that may include assessing the performance of bioprocess measurement systems and bioprocess technologies associated with monoclonal antibody production or use in biomanufacturing educational and training programs.

Description: A unit of RM 8675 consists of one vial containing approximately 1.0 mL of cryopreserved, Chinese hamster ovary K1 subtype (*Critelus griseus*; CHO-K1) lineage cell suspension with a nominal cell number of 1×10^7 cells and a time-in-culture post-clonal isolation of nine passages. The cryopreservation matrix consists of EX-CELL CD CHO Fusion culture medium supplemented with seven volume percent (7 % v/v) dimethylsulfoxide.

When in culture, cell suspensions propagated from RM 8675 are expected to produce a non-originator, humanized, IgG1κ monoclonal antibody, cNISTmAb, having the same primary amino acid sequence as the NISTmAb monoclonal antibody (RM 8671) [1]. The CHOZN® GS-/- host cell line [2] was used to develop the NISTCHO cell line. Clonality of the cell line was established by limiting dilution and confirmed using optical photomicroscopy. The development of RM 8675 was conducted with materials and reagents free of animal origin.

Non-Certified Values: The non-certified values presented in Table 1 are suitable for method development, harmonization, and process control but do not provide metrological traceability to the International System of Units (SI) or other higher order reference systems. These values represent the copy number ratio of NISTCHO-specific genes (3′ integration site, 5′ integration site, heavy chain (HC), and light chain (LC)) relative to the reference genes in the CHO-K1 genome—specifically, Fbxw2 and Slc35a1. (Note that Slc35a1 is known to be single copy (haploid), while Fbxw2 is present in three copies (triploid) [3].

The values below were derived by fitting a statistical model to digital Polymerase Chain Reaction (PCR) measurements performed on the RM 8675 materials. The expanded uncertainty is calculated in accordance with the ISO/JCGM Guide [4,5] and reflects contributions from differences among vials as well as variability introduced during sample preparation and repeated measurements. A 95 % confidence interval, expressed as Estimated Average Ratio ± Expanded Uncertainty, means that for a measurement system equivalent to the one used at NIST, the average of repeated copy number ratio measurements from a single vial will, as the number of measurements increases, converge to a value within this interval with 95 % probability.

Additional Information: Primer and probes sequences for each assay, PCR conditions, and cell viability measurements are listed in Appendix A.

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **31 May 2030.** The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Reference Material Information Sheet. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm). NIST will monitor the stability of the copy number ratios and the viability of the remaining vials at least once every 12 months. These cells and the DNA measurements are stable for long periods of time when stored as recommended [6].

Rebecca A. Zangmeister, Acting Chief
Biomolecular Measurement Division

Steven J. Choquette, Director
Office of Reference Materials

Table 1. Non-Certified Values of the Copy Number Ratios of NISTCHO Specific Genes to Reference Genes in the CHO-K1 Genome[(a)]

Target Gene	Reference Gene	Estimated Average Ratio	Expanded Uncertainty
3′	Slc35a1	3.05	0.48
3′	Fbxw2	0.97	0.12
5′	Slc35a1	3.00	0.32
5′	Fbxw2	0.956	0.062
HC	Slc35a1	3.03	0.33
HC	Fbxw2	0.966	0.069
LC	Slc35a1	3.03	0.62
LC	Fbxw2	0.96	0.15

[(a)] Expanded uncertainties have been rounded to two significant digits. Reference values have been rounded to match the number of decimal positions in the rounded expanded uncertainties.

Safety: RM 8675 is a Biosafety Level 1 material and should be handled according to applicable federal, state, and/or local regulations and according to the policies and procedures of the recipient's organization.

Storage: The original unopened vial of RM 8675 should be stored at ≤ –140 °C, such as in the vapor phase of liquid nitrogen. The vial containing RM 8675 may be permeable to liquid nitrogen and should NOT be stored submerged in it.

Use: USE OF RM 8675 IS STRICTLY LIMITED TO NON-COMMERCIAL RESEARCH USE. Additionally, RM 8675 AND ANY MATERIALS DERIVED FROM RM 8675 MAY NOT BE USED IN HUMANS OR ANIMALS, INCLUDING FOR ANY DIAGNOSTIC, PROGNOSTIC, CLINICAL OR TREATMENT PURPOSES. This cell line has been engineered by MilliporeSigma to express a non-originator version of NISTmAb [3]. This product may not be modified to express a molecule other than the NISTmAb molecule. Recipient is expected to establish one or more cryopreserved cell banks to support all use of RM 8675. (The first inoculation to shake flask culture should be counted as culture passage 10.) Recipient may refer to the CHOZN® Platform Technical Bulletin provided by Sigma-Aldrich [2] for additional recommendations and/or guidance, including the selection of culture media, conditions, and scaling for propagating RM 8675.

Homogeneity and Non-Certified Value Measurements: A total of five vials of RM 8675 were analyzed to assess homogeneity and measure non-certified values. Each cryovial was removed from liquid nitrogen storage and thawed in a 37 °C water bath with constant manual shaking for 2 min. Following thawing, the exterior of each vial was sprayed with 70 % isopropanol and transferred to the biosafety cabinet. The cell suspension was then transferred from the cryovial into a 15 mL tube containing 8 mL of pre-warmed media. This tube was centrifuged at 200 rcf for 5 min. The supernatant was removed, and the cell pellet was resuspended in 8 mL of fresh, pre-warmed EX-CELL CD CHO Fusion culture medium. From this, 1 mL was removed of which 600 µL was used for cell viability analysis. The remaining 7 mL was centrifuged at 200 rcf for 5 min. The supernatant was removed, and the cell pellet was stored at –80 °C until DNA extraction.

For DNA extraction, the frozen cell pellet was thawed and resuspended with 200 µL phosphate buffered saline (pH 7.4). The sample was then split equally (100 µL each) for use with two different DNA extraction kits: QIAGEN DNeasy Blood & Tissue Kit and New England Biolabs Monarch Spin gDNA Extraction Kit, following the respective manufacturer's instructions.

Following extraction, digital PCR (ddPCR) was performed to measure the concentration of mAb heavy chain (HC), mAb light chain (LC), the 5′ integration site, and 3′ integration site, as well as two reference genes (Slc35a1 and Fbxw2). The ddPCR was conducted using the Bio-Rad QX200 system with Bio-Rad droplet digital PCR (ddPCR) Supermix for Probes. Primer and probes sequences for each assay, as well as PCR conditions, are listed in Tables A1 and A2, respectively. The cell viability measurements for the five vials are provided in Table A3.

REFERENCES

[1] Cleveland, M. H.; Karageorgos, I. L.; Marino, J.P.; Tarlov, M.J.; Yandrofski, K.S.; Zangmeister, R.A.; Kelman, Z.; *Recommended Nomenclature Convention for the NISTCHO Cell Line and its Product Monoclonal Antibody, cNISTmAb*; Mabs.; Vol. 17 (2025).

[2] CHOZN®; https://www.sigmaaldrich.com/US/en/services/custom-products/chozn-cell-line-engineering-and-development (accessed June 2025)

[3] Dahodwala, H.; Hodzic, I.; Slesarev, A.; Cutak, B.; Kuzin, A.; Lal, R.; Liu, J.; Mahon, J.; Narasimhan, L.N.; Onuska, J; et al.; *Development and Characterization of the NISTCHO Reference Cell Line*; Biotech J.; Vol. 20, (2025).

[4] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at https://www.bipm.org/en/committees/jc/jcgm/publications (accessed June 2025); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at https://www.nist.gov/pml/nist-technical-note-1297 (accessed June 2025).

[5] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the Guide to the Expression of Uncertainty in Measurement – Propagation of Distributions Using a Monte Carlo Method*; Joint Committee for Guides in Metrology (JCGM) (2008); available at https://www.bipm.org/en/committees/jc/jcgm/publications (accessed June 2025).

[6] Babel, M.; Mamilos, A.; Seitz, S.; Niedermair, T.; Weber, F.; Anzeneder, T.; Ortmann, O.; Dietmaier, W.; Brochhausen, C.; *Compared DNA and RNA Quality of Breast Cancer Biobanking Samples After Long-term Storage Protocols in –80 °C and Liquid Nitrogen*; Sci Rep., Vol. 10 (2020).

If you use this RM in published work, please reference:

Cleveland MH, Karageorgos IL, Marino JP, Tarlov MJ, Yandrofski KS, Zangmeister, RA, Kelman Z (2025) Recommended Nomenclature Convention for the NISTCHO Cell Line and its Product Monoclonal Antibody, cNISTmAb. (Mabs.), Vol. 17.

Certain commercial equipment, instruments, or materials may be identified in this Reference Material Information Sheet to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Users of this RM should ensure that the Reference Material Information Sheet in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

* * * * * * * End of Reference Material Information Sheet * * * * * * *

APPENDIX A

Values of Potential Interest: Values of potential interest are provided in Table A1, A2 and A3. Table A1 contains the primer and probe sequences used to determine the copy number ratio of the heavy chain, light chain, 5' integration site, and 3' integration site relative to Slc35a1 and Fbxw2. For each reaction, the primers were used at a concentration of 900 nmol/L each and the probe was at a concentration of 250 nmol/L. Table A2 lists PCR thermocycling conditions and Table A3 contains cell viability measurements and cell count estimates obtained from the Vi-CELL XR Cell Viability Analyzer

Table A1. Primer and Probe sequences used for digital PCR copy number ratio determination

Heavy Chain (HC)	
Forward Primer	GACCTACATCTGCAACGTGA
Reverse Primer	GTTCCACTCTCTTGTCGACC
Probe	[6FAM] ACCACAAGCCCAGCAACACC [BHQ1]
Light Chain (LC)	
Forward Primer	GCGAGGCTAAAGTGCAATGG
Reverse Primer	GTCCTTGGAGTCCTGTTCCG
Probe	[6FAM] CACTGCAGTCGGGGAATTCC [BHQ1]
5' Integration Site	
Forward Primer	TCTCTGACAGTGACAGACGC
Reverse Primer	CAGCCATCTGTTGTTTGCCC
Probe	[FAM] GGAAAGGACAGTGGGAGTGG [BHQ1]
3' Integration Site	
Forward Primer	CGCGTAATCTGCTGCTTGC
Reverse Primer	TGTGCACATTCACAATGCTGC
Probe	[FAM] GCGAAGGGAGAGAAACCACT [BHQ1]
Fbxw2	
Forward Primer	ACAGAGAGCGTGATTAGCCG
Reverse Primer	TACTGTGGTCAGGCATGCTG
Probe	[FAM] GGACTGGATGGGCACAATGA [BHQ1]
Slc35a1	
Forward Primer	TTGGAGGCCTCTACACTTCT
Reverse Primer	AGCATCACTGAAGCAATGGT
Probe	[FAM] AAAGGCTTCTCAGCAGCTGCAGCCA [BHQ1]

Table A2. PCR thermocycling conditions

Stage	Step	PCR Conditions	Cycles
1	1	95 °C for 10 min	1
2	1	94 °C for 30 s	40
	2	59 °C for 1 min	
3	1	72 °C for 10 min	1

Table A3. Vi-CELL values obtained for five vials of RM 8675

Vial Number	Viability (%)	Viable Cell Count	Estimated Viable Cell Count in each 1 mL vial
4	97	1.42E+06	1.14E+07
82	95.7	1.38E+06	1.10E+07
243	96.9	1.41E+06	1.13E+07
316	97.1	1.54E+06	1.23E+07
406	97.6	1.41E+06	1.13E+07

* * * * * * * * * * End of Appendix A * * * * * * * * * *