

NISTCHO Test Material, Clonal CHO-K1 Cell Line Producing cNISTmAb Guidance Document

NIST Research-Grade Test Material 10197

Purpose: NIST Research-Grade Test Material (RGTM) 10197 is being provided on a collaborative, no-cost basis for recipients to evaluate potential fitness-for-purpose as a reference material by measuring properties of RGTM 10197 and/or by measuring bioprocesses containing RGTM 10197. Material shall only be used in accordance with TERMS AND CONDITIONS FOR TRANSFER OF NIST RGTM 10197 accepted when requesting the material.

Description: A unit of RGTM 10197 consists of one vial of cryopreserved, Chinese hamster ovary K1 subtype (Sp: *Critellus griseus*; CHO-K1) lineage cell suspension with nominal volume of 1.0 mL, nominal cell number of 1.3×10^7 cells and a time-in-culture post-clonal isolation of 6 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 RGTM 10197 are expected to produce a non-originator, humanized, IgG1 κ monoclonal antibody, cNISTmAb, having the same primary amino acid sequence as the NISTmAb monoclonal antibody.

Period of Use: Recipient may use RGTM 10197 from receipt until the earlier of either completion of the evaluation or the termination date, November 16, 2026, provided in the TERMS AND CONDITIONS FOR TRANSFER OF NIST RGTM 10197 accepted when requesting the material.

Reporting of Results: As of the date of issue of this document, shown above, recipient should report results by email to nistcho@nist.gov in accordance with TERMS AND CONDITIONS FOR TRANSFER OF NIST RGTM 10197 accepted when requesting the material.

Safety: RGTM 10197 AND ANY MATERIALS DERIVED FROM RGTM 10197 MAY NOT BE USED IN HUMANS OR ANIMALS, INCLUDING FOR ANY DIAGNOSTIC, PROGNOSTIC, CLINICAL OR TREATMENT PURPOSES. Users of RGTM 10197 should review the Safety Data Sheet Exemption Letter provided with this material and available at https://shop.nist.gov/ccrz__ProductDetails?sku=10197. RGTM 10197 is a Biosafety Level 1 material and should be handled according to applicable federal, state, and/or local regulations and according to policies and procedures of recipient's organization.

Storage: The original unopened vial of RGTM 10197 should be stored at ≤ -140 °C, such as in the vapor phase of liquid nitrogen. Units of RGTM 10197 as provided may be permeable to liquid nitrogen and **should not** be stored submerged in liquid nitrogen.

Instructions for Use: Initial propagation and banking: Recipient is expected to establish one or more cell banks cryopreserved at culture passage 9 post-clonal isolation to support all use of RGTM 10197. (The first inoculation to shake flask culture should be counted as culture passage 7.) Recipient may wish to refer to the CHOZN[®] Platform Technical Bulletin provided by Sigma-Aldrich for additional recommendations and/or guidance, including the selection of culture medias, conditions, and scaling for propagating RGTM 10197.

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Disclaimer: *Certain commercial equipment, instruments, or materials may be identified in this 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.

NIST Additional Information

Cell line lineage and development: The CHOZN[®] GS^{-/-} host cell line 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 the RGTM 10197 was conducted with materials and reagents free of animal origin (AOF).

Propagation and cell banking: The NISTCHO cell line was propagated by NIST to prepare a cryopreserved cell bank, units of which are being made available as RGTM 10197. The propagation and cryopreservation of RGTM 10197 was conducted with materials and reagents free of animal origin (AOF).

Adventitious contaminants: Three units of the RGTM 10197 lot from the beginning, middle and end of cell banking have been assayed for bacterial and fungal contamination by direct inoculation with no contamination apparent. Three units of the RGTM 10197 lot, also from the beginning, middle and end of cell banking, have been assayed by PCR amplification for contamination with mycoplasma, contamination with select adventitious viruses, and contamination with human or mouse DNA sequences with no contamination apparent.

Within lot homogeneity: Eight units of the RGTM 10197 lot, sampled from across the cell banking process, have been assayed for recoverable viability at 24 hours post thaw with all units exceeding an assay-specific “pass” threshold. In addition, the cultures established from each of the eight units were successfully maintained through logarithmic growth to stationary culture phase as assayed by viable cell density determination. These data are presented graphically in Figures 1 to 2 for information purposes only.

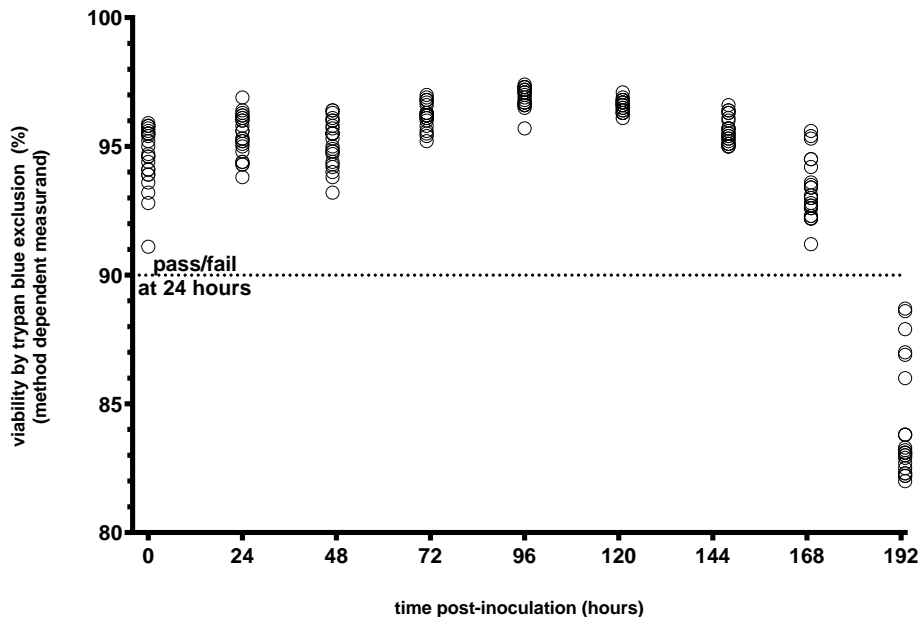


Figure 1. Viability ratios used for within-lot homogeneity determination of RGTM 10197. For each time point, 24 values are plotted reflecting the range of values observed for 8 cultures, each established from a unique unit of the RGTM 10197 lot, measured in technical triplicate.

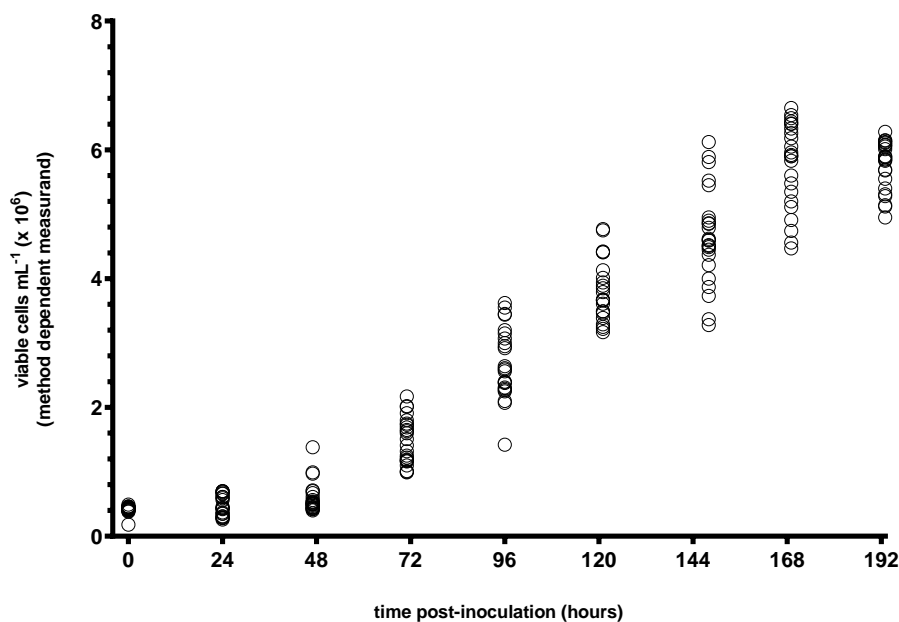


Figure 2. Viable cell densities used for within-lot homogeneity determination of RGTM 10197. For each time point, 24 values are plotted reflecting the range of values observed for 8 cultures, each established from a unique unit of the RGTM 10197 lot, measured in technical triplicate.

Suggested nomenclature system for identification and disambiguation of NISTCHO cell line-derived materials: NIST RGTM 10197 NISTCHO Test Material is an atypical artifact compared with others provided by NIST in that RGTM 10197 is living and will grow and change during use. In fact, reviving the RGTM 10197 artifact as received to establish viable cultures and a laboratory's own working cell banks (WCBs) introduces changes that may impact the comparability of materials between laboratories.

Further, the underlying cell substrate recombinantly overexpresses an IgG1 κ monoclonal antibody product that has the same primary amino acid sequence as NIST RM 8671 NISTmAb. The produced antibody product is considered a "non-originator" version of RM 8671 and is, therefore, *neither* NIST RM 8671 *nor* NISTmAb. As such, the antibody product derived from NIST RGTM 10197 must in all instances be referred to as "cNISTmAb" to designate an origin from a Chinese hamster ovary (CHO) cell substrate.

Given these considerations, NIST suggests that recipients of NIST RGTM 10197, when publicly presenting or reporting methods, materials, results, *etc.* from use of the material, consider Tables B1 to B2 below for a harmonized nomenclature format for protein product, cell substrates (WCBs), cultures (batches), purifications (lots), and potential derived materials. While elaborate, use of identifiers such as these among NISTCHO users will add significant value by facilitating (ideally) unambiguous *identification* of any tangible material or process and reasonable *traceability* of any material or process to the original NIST artifact. The format of each identifier has been developed to reflect potential sources for significant change including: cell culture media system in use, culture passage number of starting cell substrate, and source organization of the material.

While these identifiers cannot feasibly represent all significant details for a material or process, NIST's goal is that this nomenclature system will serve as a starting point with utility, and NIST welcomes feedback and suggestions from individual recipients and from the community at large.

Table 1. Suggested identifier format and examples for NISTCHO protein products, cell substrates (WCBs), cultures, and purifications.

| <i>Protein product identifier</i> | |
|--|---|
| Format: | cNISTmAb/[source organization] |
| Example: | cNISTmAb/NIST |
| <i>Cell substrate (WCB) identifier</i> | |
| Format: | [NIST RGTM/RM number]/[passage number]/[media system]/[source organization]/[serial number] |
| Example: | 10197/p09/EX-CELL [®] CD CHO/NIST/1 |
| <i>Culture (batch) identifier</i> | |
| Format: | [media system]/[date of production culture start]/[serial number] |
| Example: | EX-CELL [®] CD CHO/2023-04-15/1 |
| <i>Purification (lot) identifier</i> | |
| Format: | [date of purification start]/[serial number] |
| Example: | 2023-04-30/1 |

Table 2. Combined identifier examples.

| <i>Combined identifier example for a culture (batch)</i> | |
|--|---|
| Cell substrate (WCB) identifier | 10197/p09/EX-CELL [®] CD CHO/NIST/1 |
| Culture (batch) identifier | EX-CELL [®] CD CHO/2023-04-15/1 |
| <i>Combined identifier example for bulk or aliquoted purified protein product</i> | |
| Protein product identifier | cNISTmAb/NIST |
| (formulation details) | 10 mg mL ⁻¹ ; 25 mmol L ⁻¹ histidine·HCl pH 6.0 |
| Cell substrate (WCB) identifier | 10197/p09/EX-CELL [®] CD CHO/NIST/1 |
| Culture (batch) identifier | EX-CELL [®] CD CHO/2023-04-15/1 |
| Purification (lot) identifier | 2023-04-30/1 |
| <i>Combined identifier examples for cell substrate- or process-derived materials</i> | |
| Material description | total cell protein extract/NIST |
| Cell substrate (WCB) identifier | 10197/p09/EX-CELL [®] CD CHO/NIST/1 |
| Culture (batch) identifier | EX-CELL [®] CD CHO/2023-04-15/1 |
| Material description | genomic DNA/NIST |
| Cell substrate (WCB) identifier | 10197/p09/EX-CELL [®] CD CHO/NIST/1 |
| Culture (batch) identifier | EX-CELL [®] CD CHO/2023-04-15/1 |
| Material description | conditioned culture media/NIST |
| Cell substrate (WCB) identifier | 10197/p09/EX-CELL [®] CD CHO/NIST/1 |
| Culture (batch) identifier | EX-CELL [®] CD CHO/2023-04-15/1 |

Revision History: 16 February 2023 (Added instructions for counting culture passages to section “Instructions for Use. Changed statement in section “Safety” to recommend Biosafety Level 1 procedures.); **06 January 2023** (Original version date.)