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RNA Encapsulated in Lipid Nanoparticles Guidance Document

NIST Research-Grade Test Material 10240

The material being provided is a NIST Research Grade Test Material (RGTM). This material is not a NIST Standard Reference Material® or a NIST Reference Material. NIST RGTMs are described in the latest edition of NIST Special Publication 260-136.

Purpose: NIST Research-Grade Test Material (RGTM) 10240 is being provided on a collaborative, no-cost basis for recipients to evaluate potential fitness for purpose as a reference material for the standardization of measurement techniques used in the characterization of lipid nanoparticle (LNP)/RNA formulations. Attributes of interest could include RNA copy number concentration, RNA encapsulation efficiency, LNP diameter and diameter distribution, and particle number concentration. Suitable methods could include, but are not limited to: RT-dPCR/qPCR for RNA copy number concentration; fluorescent dye-binding assays for encapsulation efficiency; and static and dynamic light scattering (SLS/DLS), small-angle X-ray/neutron scattering (SAXS/SANS), field flow fractionation coupled to multi-angle light scattering (AF4-MALS), and nanoparticle tracking analysis (NTA) for LNP diameter, diameter distribution, or particle number concentration.

Description: This RGTM is designed to be similar to mRNA vaccines and is composed of a synthetic RNA encapsulated in Lipid Nanoparticles (LNPs). A unit of RGTM 10240 consists of two components: Part 1, labeled "Full LNPs (with RNA)" and Part 2, labeled "Empty LNPs (without RNA)". Four vials of Part 1 (100 µL each) and one vial of Part 2 (200 µL) are included in a unit.

Period of Use: Recipient may use RGTM 10240 from receipt until the earlier of either completion of the recipient's participation in the evaluation of the material's fitness-for-purpose or the material's nominal expiration date, 01 January 2027. Note that long-term stability is still under evaluation, and this expiration date is subject to revision, which could include either shortening or lengthening.

Reporting of Results: Please return comments and feedback about your experience with RGTM 10240 by email to LNPs@nist.gov no later than 60 days after the material is received.

Instructions for Use: Thaw the tube on ice. Once thawed, vortex briefly and gently, centrifuge briefly, and repeat. The material should be used immediately upon thawing and should not be frozen again.

Safety: RGTM 10240 is FOR RESEARCH USE. It should be handled as a Biosafety Level 1 material, according to applicable federal, state, and/or local regulations, and according to the policies and procedures of the recipient's organization.

Storage: The material is shipped on dry ice and should be stored frozen at –80 °C. Transfers from dry ice to freezer should be performed as quickly as possible due to the small sample volumes and possibility of rapid thawing.

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NIST Additional Information

Part 1 (Full LNPs) consists of a mixture of RNA and four different lipids formulated into LNPs using microfluidic assembly methods. The components are supplied in a buffered solution consisting of 10.0 mmol/L MOPS, 4.0 mmol/L NaOH, 75.0 mmol/L NaCl, and glycerol at a volume fraction of 20.0 %. The lipids consist of 1,2-dioleyloxy-3-dimethylaminopropane (DODMA), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2k) at mol ratios of approximately 50:10:38.5:1.5 respectively, and a total mass concentration (sum of all four lipid concentrations) between approximately (0.1 to 20) mg/mL. The mass-weighted average particle diameter is expected to be in the range of approximately (50 to 300) nm, and particle concentration within the range of (10^9 to 10^{13}) g^{-1}. The RNA is a non-infectious, synthetic, in vitro transcribed fragment containing a nucleotide sequence matching positions 25949-29698 of the USA-WA1/2020 isolate of SARS-CoV-2. The RNA mass concentration is approximately (5 to 1000) µg mL^{-1}, corresponding to a copy number concentration of approximately (10^9 to 10^{11}) $µL^{-1}$. The genetic sequence information of the RNA is listed in the "NIST Additional Information" section of this document.

Part 2 (Empty LNPs) has been prepared using the same nominal lipid concentrations and process parameters as Part 1, but with the RNA omitted. Nevertheless, the exact lipid concentrations, as well as other properties such as particle diameter and particle concentration, have not been evaluated and may differ from Part 1. Part 2 has been produced using the same microfluidic equipment as Part 1. Although this equipment is cleaned between uses, it is possible that low levels of the RNA may still be found in this nominally "empty" material by sensitive techniques such as digital PCR (dPCR).

RNA Sequence

3984 bases total

Black letters correspond to GenBank accession MN_985325.1, nucleotides 25949-29698
Blue letters correspond to sequence tags

GGGAGAAUCCUCACACUUUGUCUCUACUGCAUACUCAGUAAUUAAUUUCCAAGACACCUGCUUUGUUUCUUCAGGCUCUUCUGGGU
UAAAUUGGUGGUUAUACUGAAAAAUGGGAAUCUGGAGUAAAAGACUGUGUUGUAUUACACAGUUACUUCACUUCAGACUAUUACCA
GCUGUACUCAACUCAAUUGAGUACAGACACUGGUGUUGAACAUGUUACCUUCUUCAUCUACAAUAAAAUUGUUGAUGAGCCUGAAG
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ACUAGCGUGCCUUUGUAAGCACAAGCUGAUGAGUACGAACUUAUGUACUCAUUCGUUUCGGAAGAGACAGGUACGUUAAUAGUUAA
UAGCGUACUUCUUUUUCUUGCUUUCGUGGUAUUCUUGCUAGUUACACUAGCCAUCCUUACUGCGCUUCGAUUGUGUGCGUACUGCU
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