

H5N1 (Avian Influenza) Synthetic RNA Fragments

Guidance Document

NIST Research-Grade Test Material 10263

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) 10263 is being provided on a collaborative basis for recipients to evaluate the material's potential fitness-for-purpose as a reference material for RT-qPCR assay development and evaluation, calibration of RT-qPCR methods and benchmarking other H5N1 control materials. For more details, please visit <https://www.nist.gov/programs-projects/h5-influenza-positive-controls>.

Description: A unit of RGTM 10263 consists of three components labeled as follows Part A: H5_HA, Part B: N1_NA, Part C: MP. Each component contains approximately 100 µL of target RNA in a background of 5 ng/µL Jurkat RNA in a buffered solution. All target RNA sequences match the A/American Wigeon/South Carolina/22/2021 CVV reassortant virus strain. The target RNA for Part A contains sequence from the H5 hemagglutinin gene, the target RNA for Part B contains sequence from the N1 neuraminidase gene, and the target RNA for Part C contains sequence from the matrix protein gene. The genetic sequence information of the target RNA for each part is listed in the "NIST Additional Information" section of this document.

Period of Use: Recipient may use RGTM 10263 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 of November 1, 2029.

Reporting of Results: Please return comments and feedback about your experience with RGTM 10263 by email to h5flu@nist.gov no later than 60 days after the material is received.

Safety: RGTM 10263 is FOR RESEARCH USE. This is a human source material. RGTM 10263 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 material should be stored frozen at -80 °C.

Instructions for Use: Thaw the tube at room temperature. Once thawed, vortex briefly, centrifuge briefly and repeat. Note: multiple freeze-thaws of the material may result in lower concentration estimates.

NIST Technical Contact(s): Megan Cleveland, megan.cleveland@nist.gov, 301-975-5473

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

Nominal concentration values (Table 1) were obtained using reverse transcription digital polymerase chain reaction (RT-dPCR) with the Bio-Rad One Step RT-ddPCR Advanced Kit for Probes (catalog # 1864021) on the QX200 system. The primer and probe sequences for each assay are listed in Table 2.

Table 1. Target RNA Concentrations

Part	Target RNA	Nominal Concentration (copies/ μ L)
A	H5_HA	9.1×10^5
B	N1_N5	8.4×10^5
C	MP	6.6×10^5

Table 2. Primer and Probe Sequences for RT-dPCR Assays (5' – 3')

HA-2	Forward Primer	ATCCCAAGTAAACGGGCAAC
	Reverse Primer	TGTTGCAGTGGCCATATTCC
	Probe	ACCAGATGATGCAATCCATTTCGAGA
NA-2	Forward Primer	GTCCAGACAATGGAGCTGTG
	Reverse Primer	CGCATCAGGATAACAGGAGC
	Probe	CAAGCAATGGGCAGGCCTCA
InfA	Forward Primer	CAAGACCAATCYTGTACCTCTGAC
	Reverse Primer	GCATTYTGGACAAAVCGTCTACG
	Probe	CGTGCCCAGTGAGCGAGGACTGCA

Appendix A. Genetic Sequence Information

Part A: H5_HA

GGTTCACTCTGTCAAAATGGAGAACATAGTACTACTTCTTGCAATAGTTAGCCTTGTTAA
AAGTGATCAGATTTGCATTGGTTACCATGCAAACAATTCGACAGAGCAAGTTGACACGAT
AATGGAAAAGAACGTCACCTGTTACACATGCCCAAGACATACTGGAAAAACACACAACGG
GAAGCTCTGTGATCTAAATGGGGTGAAGCCTCTGATTTTAAAGGATTGTAGTGTAGCTGG
ATGGCTCCTCGGAAACCCAATGTGCGACGAATTCATCAGAGTGCCTGAATGGTCCTACAT
AGTGGAGCGGGCTAACCCAGCTAATGACCTCTGTTACCCAGGGAGCCTCAATGACTATGA
AGAACTGAAACACATGTTGAGCAGAATAAATCATTTTGAGAAGATTCTGATCATCCCCAA
GAGTTCCTGGCCAAATCATGAAACATCACTAGGGGTGAGCGCAGCTTGTCCATACCAGGG
AGCGCCCTCCTTTTTTCAGAAATGTGGTGTGGCTTATCAAAAAGAACGATGCATACCCAAC
AATAAAGATAAGCTACAATAATACCAATCGGGAAGATCTCTTGATACTGTGGGGGATTCA
TCATTCCAACAATGCAGAAGAGCAGACAAATCTCTACAAAACCCAACCACCTACATTTTC
AGTTGGAACATCAACTTTAAACCAGAGGTTGGCACCAAAAATAGCTACTAGATCCCAAGT
AAACGGGCAACGTGGAAGAATGGACTTCTTCTGGACAATCTTAAACCAGATGATGCAAT
CCATTTTCGAGAGTAATGGAAATTTTATTGCTCCAGAATATGCATACAAAATTGTCAAGAA
AGGGGACTCAACAATTATGAAAAGTGGAGTGGGAATATGGCCACTGCAACACCAAATGTCA
AACCCAGTAGGTGCGATAAATTCTAGTATGCCATTCCACAACATACATCCTCTCACCAT
TGGGGAATGCCCCAAATACGTGAAGTCAAACAAGTTGGTCCTTGCGACTGGGCTCAGAAA
TAGTCCTCTACGAGAAACGCGAGGCCTGTTTGGGGCGATAGCAGGGTTTATAGAGGGAGG
ATGGCAGGGAATGGTTGATGGTTGGTATGGGTACCATCATAGCAATGAGCAGGGGAGTGG
GTACGCTGCGGACAAAGAATCCACCCAAAAGGCAATAGATGGAGTTACCAATAAGGTCAA
CTCAATCATTGACAAAATGAACACTCAATTTGAGGCAGTTGGAAGGGAGTTTAATAACTT
AGAAAGGAGGATAGAGAATTTGAACAAGAAAATGGAAGACGGATTCCTAGATGTCTGGAC
CTATAATGCTGAACTTCTAGTTCTCATGGAAAACGAGAGGACTCTAGATTTCCATGATTC
AAATGTCAAGAACCTTTACGACAAAGTCAGATTACAGCTTAGGGATAATGCAAAGGAGCT
GGGTAACGGCTGTTTCGAATTCTATCACAATGTGATAATGAATGTATGGAAAGTGTGAG
AAATGGGACGTATGACTACCCTCAGTATTCAGAAGAAGCAAGATTAAAAAGAGAAGAAAT
AAGCGGAGTGAAATTAGAATCAGTAGGAACCTACCAGATACTGTCAATTTATTCAACAGC
GGCAAGTTCCCTAGCACTGGCAATCATGATGGCTGGTCTATCTTTATGGATGTGCTCCAA
TGGGTCGTTACAGTGCAGAATTTGCATTTAGATTTGTGAGCTCAGATTGTAGTTAAAAAC
AC

Part B: N1_NA

AGTTCAAAATGAATCCAAATCAAAAAGATAACAACCATTGGATCAATCTGTATGGTAATTG
GGATAGTCAGCTTGATGCTGCAAATTGGGAACATAATCTCAATATGGGTAGCCATTCAA
TCCAAACAGGGAACCAATACCAGCCTGAACCATGCAATCAAAGCATCATTACCTATGAGA
ACAACACCTGGGTAAATCAGACGTATGTCAACATCAGCAATACCAATTTTCTTGCTGAGC
AGGCTGTTACTTCGGTAACATTAGCGGGCAATTCATCTCTTTGCCCTATTAGTGGGTGGG
CAATATACAGTAAGGACAACGGTATAAGAATTGGGTCCAAGGGGGATGTGTTTGTATATA
GAGAACCGTTCATCTCATGCTCCCACTTGGAATGCAGAACCTTTTCTGACCCAGGGAG
CTCTGCTGAATGACAAACATTCTAATGGGACCGTTAAGGATAGAAGCCCTTATAGAACTT
TGATGAGTTGTCCCGTGGGTGAGGCTCCTTCCCGGTACAATTCAAGATTTGAGTCTGTTG
CTTGCTCGGCAAGTGCTTGTCTGATGGCATCAGTTGGTTGACAATCGGTATTTCTGGTC
CAGACAATGGAGCTGTGGCTGTATTGAAGTACAATGGCATAATAACGGATACTATCAAGA
GTTGGAGAAACAACATTTTGAGAACTCAAGAATCTGAATGTGCGTGCGTAAATGGCTCTT
GCTTCACCGTAATGACTGATGGACCAAGCAATGGGCAGGCCTCATATAAAATCTTCAAGA
TAGATAAAGGGAAAGTTGTCAAATCAGTTGAATTGAATGCCCTAATTACCACTACGAGG
AATGCTCCTGTTATCCTGATGCGGGTGATATTATGTGTGTGTGCAGGGACAATTGGCATG
GCTCAAACCGGCCGTGGGTATCTTTAATCAAAATCTGGAGTATCAAATAGGATATATAT
GCAGTGGGGTTTTCGGGGACAATCCCCGCCCAATGATGGAACAGGCAGTTGCAGTCCAA
TGTCTCTAATGGGGCATATGGGGTAAAAGGGTTTTCATTTAAGTACGGTAATGGGGTTT
GGATCGGAAGAACAAAAAGCACTAGTTCAGAAAGCGGCTTTGAGATGATTTGGGATCCGA
ATGGGTGGACTGAGACGGACAGTAGTTTCTCAGTGAAGCAAGACATTGTAGAAATAACTG
ACTGGTCAGGATATAGTGGGAGTTTTGTCCAGCATCCAGAACTGACAGGATTAGATTGCA
TGAGGCCTTGTTTCTGGGTGAGCTAATTAGAGGGAGGCCCAAAGAGAACACAATTTGGA
CTAGCGGGAGCAGCATATCCTTTTGTGGTGTAAATAGTGACACTGTGGGTGGTCTTGCG
CAGACGGTGCTGAGTTGCCATTACCAATTGACAAGTAGTTTGTTCAAAAAACT

Part C: MP

ATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCTCTCTATCGTCCCGTCGGGCCCCCTCAAAGCCGAG
ATCGCGCAGAGACTTGAAGATGTCTTTGCAGGGAAGAACACCGATCTTGAGGCTCTCATGGAATGGCT
AAAGACAAGACCAATCCTGTACCTCTGACTAAGGGGATTTTGGGATTTGTGTTACGCTCACCGTGC
CCAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCTCTAAATGGAAATGGAGACCCAAA
CAACATGGACAGGGCAGTCAAGTTGTACAGGAAATTGAAGAGAGAGATAACATTCCATGGGGCTAAA
GAAGTTGCACTCAGTTACTCAACCGGTGCACTTGCCAGTTGTATGGGTCTCATATACAACAGGATGGG
GACGGTGACCGCAGAAAGTGGCATTGGGCCTAGTGTGTGCCACCTGTGAGCAGATTGCTGATTCACAGC
ATCGGTCTCACAGACAGATAGCTACCACCACCAACCCACTAATCAGACATGAAAACAGAATGGTGTG
GCCAGTACTACAGCTAAGGCTATGGAGCAGATGGCTGGATCGAGTGAGCAAGCAGTGGAAGCCATGG
AGGTTGCTAGTCAGGCTAGGCAGATGGTGCAGGCGATGAGGACCATTGGAACCTATCCTAGCTCCAGT
GCCGGTCTGAGAGATGATCTCCTTGAAAATTTGCAGGCCTACCAAAAACGGATGGGAGTGCAACTGCA
GCGATTCAAGTGATCCTCTCGTTATTGCCGCAAGTATCATTGGGATCTTGCACTTGATATTGTGGATTCT
TTGATCGCCTTTTCTTCAAATGCGTTTATCGTCGCCTTAAATACGGTTTGAAAGGAGGGCCTTCTACGG
AAGGAGTACCTGAGTCCATGAGGGAAGAGTACCGGCAGGAACAGCAGAGTGCTGTGGATGTTGACGA
TGGTCATTTTGTCAACATAGAGCTGGAGTAA