# SARS-CoV-2 Synthetic RNA Fragments Guidance Sheet

*Research Grade Test Material ID: 10169*

## Sample Description

Each unit of RGTM 10169 consists of two tubes each containing a unique synthetic RNA fragment from the SARS-CoV-2 genome. Each tube contains approximately 110 µL of the material in a background of 5 ng/µL human Jurkat RNA (stored at -80 °C, BSL-1). The concentration is approximately 5 x $10^6$ copies/µL as estimated by multiple digital PCR assays (RT-dPCR). Table 1 below lists assay-specific concentration estimates as measured by RT-dPCR.

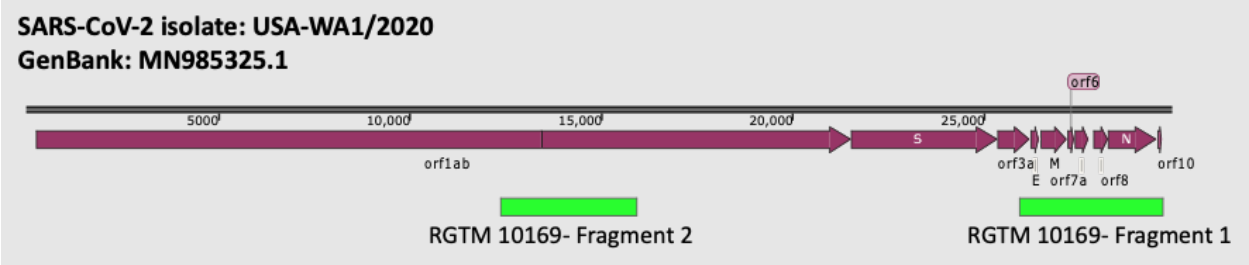


*Figure 1. Schematic of RGTM 10169 synthetic RNA fragments*

- Fragment 1: Total length: 3985 nt, Includes SARS-CoV-2 sequence: 25949-29698 of isolate USA-WA1/2020

- Fragment 2: Total length: 3790 nt, Includes SARS-CoV-2 sequence: 12409-15962 of isolate USA-WA1/2020

## Purpose

- To aid in evaluation and development of new and existing RT-qPCR assays.
- To understand SARS-CoV-2 analytical assay performance with a synthetic RNA material.
- To calibrate RT-qPCR methods and to benchmark/compare other SARS-CoV-2 controls/materials.

## Reply Date

Your feedback regarding use of this material will help us further test, develop, and improve the materials for future development. Please return your feedback survey by

**August 31, 2020**. After that date, please contact us using the information below to determine whether responses are still being collected and the material still stable.

## How to Reply

A survey link will be provided on the RGTM 10169 resource page here: https://www.nist.gov/programs-projects/sars-cov-2-research-grade-test-material

## NIST Contacts

Megan Cleveland
Megan.Cleveland@nist.gov

Peter Vallone
Peter.Vallone@nist.gov

## Storage

The material should be stored frozen at -80 °C.

## Notice and Warnings

Please review the Safety Data Sheet provided with this material. The Safety Data Sheet can also be downloaded from: https://www.nist.gov/programs-projects/sars-cov-2-research-grade-test-material

## 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 Additional Information

Supplemental data are available at: https://github.com/usnistgov/RGTM10169

## Table 1

Median concentration values (copies/microliter) as measured by RT-dPCR assays. Note: "Assay Name" is an internal designation used by NIST and not intended to align nor conflict with alternative assay naming conventions.

| Fragment 1 | Assay Name | Target Region | Median concentration (copies/microliter) |
|---|---|---|---|
| | China N | N gene | 2.4E+06 |
| | Japan | N gene | 4.5E+06 |
| | N1 | N gene | 2.2E+06 |
| | N2 | N gene | 4.6E+06 |
| | N3 | N gene | 4.5E+06 |
| | Thai | N gene | 1.9E+06 |
| | Sarbeco E | E gene | 5.4E+06 |

| | | | |
|---|---|---|---|
| Fragment 2 | Assay Name | Target Region | Median concentration (copies/microliter) |
| | China ORF1ab | ORF1ab | 5.2E+06 |
| | Pasteur 1 | ORF1ab | 5.3E+06 |
| | Pasteur 2 | ORF1ab | 5.1E+06 |
| | RdRp | ORF1ab | 4.1E+05 |

## Tables 2 and 3

RT-dPCR assay primers and information. The RT-dPCR assays were developed and optimized using primer and probe sequences from: https://github.com/usnistgov/RGTM10169/blob/master/whoinhouseassays_June_25_2020.pdf. Note: "Assay Name" is an internal designation used by NIST and not intended to align nor conflict with alternative assay naming conventions.

### Table 2: Fragment 1

| **Assay Developer** | **Assay Name** | **Primer and Probe Sequences** | **Alternate assay names** |
|---|---|---|---|
| Centers for Disease Control, China | China N | F- ggggaacttctcctgctagaat<br>P- ttgctgctgcttgacagatt<br>R- cagacattttgctctcaagctg | Centers for Disease Control, China, novel coronavirus Target 2 |
| National Institute of Infectious Diseases, Japan | Japan | F- aaattttggggaccaggaac<br>P- atgtcgcgcattggcatgga<br>R- tggcagctgtgtaggtcaac | National Institute of Infectious Diseases, Japan, NIID_2019-nCoV_N |
| United States Centers for Disease Control | N1 | F- gaccccaaaatcagcgaaat<br>P- accccgcattacgtttggtggacc<br>R- tctggttactgccagttgaatctg | United States Centers for Disease Control, 2019-nCoV N1 |
| United States Centers for Disease Control | N2 | F- ttacaaacattggccgcaaa<br>P- acaatttgcccccagcgcttcag<br>R- gcgcgacattccgaagaa | United States Centers for Disease Control, 2019-nCoV N2 |
| United States Centers for Disease Control | N3 | F- gggagccttgaatacaccaaaa<br>P- aycacattggcacccgcaatcctg<br>R- tgtagcacgattgcagcattg | United States Centers for Disease Control, 2019-nCoV N3 |

| | | | |
|---|---|---|---|
| World Health Organization | Sarbeco E | F- acaggtacgttaatagttaatagcgt<br>P- acactagccatccttactgcgcttcg<br>R- atattgcagcagtacgcacaca | World Health Organization, n-CoV Sarbeco-E |
| Ministry of Public Health, Thailand | Thai | F- cgtttggtggaccctcagat<br>P- caactggcagtaacca<br>R- ccccactgcgttctccatt | Ministry of Public Health, Thailand , 2019-nCoV |

Table 3: Fragment 2

| Assay Developer | Assay Name | Primer and Probe Sequences | Alternate assay names |
|---|---|---|---|
| Centers for Disease Control, China | China ORF1ab | F- ccctgtgggttttacacttaa<br>P- ccgtctgcggtatgtggaaaggttatgg<br>R- acgattgtgcatcagctga | Centers for Disease Control, China, novel coronavirus Target 1 |
| Pasteur Institute | Pasteur 1 | F- atgagcttagtcctgttg<br>P- agatgtcttgtgctgccggta<br>R- ctccctttgttgtgttgt | Pasteur Institute, COVID-19 Target 1, IP2 |
| Pasteur Institute | Pasteur 2 | F- ggtaactggtatgatttcg<br>P- tcatacaaaccacgccagg<br>R- ctggtcaaggttaatatagg | Pasteur Institute, COVID-19 Target 2, IP4 |
| World Health Organization | RdRp | F- gtgaratggtcatgtgtggcgg<br>P- caggtggaacctcatcaggagatgc<br>R- caratgttaaasacactattagcata | World Health Organization, n-CoV RdRp |