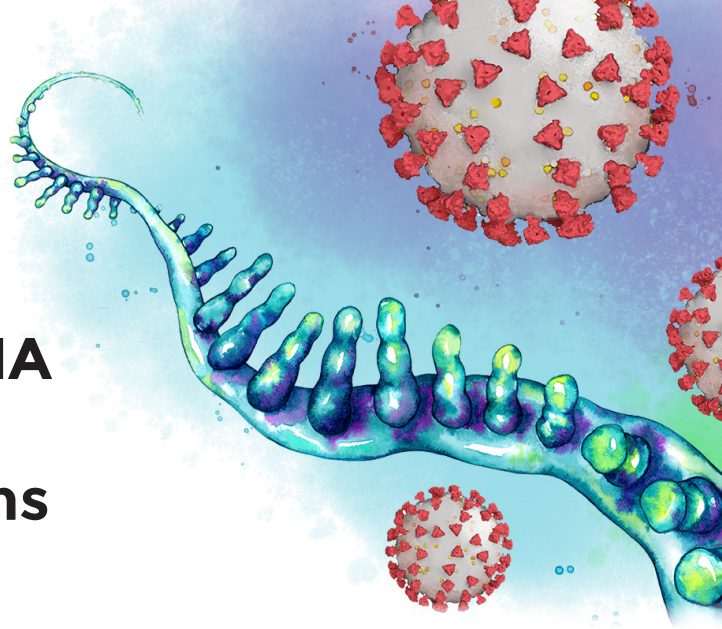


Real-Time PCR for Viral RNA Detection on Biomark HD

Frequently Asked Questions



1 Real-Time PCR for Viral RNA Detection Protocol

How was the protocol developed?

The protocol was adapted from the *Instructions for Use for the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel* for use with our microfluidic solutions (CDC-006-0019, Revision: 03).

How much sample input is required for the protocol?

5 μ L of RNA is used as input for the RT-PCR step.

Is this a one-step or two-step RT-qPCR method?

The protocol utilizes a two-step method: In the first step, reverse transcription and preamplification are done in plates. In the second step, preamplified cDNA and assay mixes are loaded onto the IFC for qPCR on Biomark.

Was this protocol tested with clinical samples?

No. The protocol was performed with SARS-CoV-2 synthetic RNA spiked into background genomic DNA.

What Fluidigm components are used in the protocol?

- **Instrument:** Biomark or Biomark HD
- **IFC:** 192.24 Dynamic Array IFC for Gene Expression (PN 100-6266)
- **IFC controller:** The IFC can be loaded on Juno™ with an RX Interface Plate (PN 101-6114) or the stand-alone IFC Controller RX (IFC-RX).



Are standard and fast PCR modes enabled with the protocol?

The standard PCR protocol is enabled on both Biomark and Biomark HD. Biomark HD is also compatible with the fast PCR protocol.

Are other third-party reagents required by the protocol?

Customers will need to acquire TaqMan® Gene Expression Master Mix from Thermo Fisher Scientific™ or another supplier. The product referenced in our protocol is the 5 mL reagent, PN 4369016.

If utilizing the fast PCR protocol, customers will need to purchase TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific, PN 4444557).

What is the limit of detection (LOD) enabled by the protocol?

The LOD is 1 copy per μ L (5 copies total per reaction).

What is the dynamic range for detection?

A dynamic range of 5 logs was achieved across all tests.

What is the estimated protocol time?

The times listed below are inclusive of RT-PCR, preamplification and protocol times on Juno, the IFC controller and Biomark:

- **Standard PCR:** ~4 hr 20 min
- **Fast PCR:** ~3 hr 20 min

Note that these estimates do not include hands-on time. Total workflow time will vary based on lab personnel or the use of automated liquid handling systems.

2 Fluidigm Consumables

What Fluidigm consumables will I need for the protocol?

Product Name	Part Number
192.24 Dynamic Array™ IFC for Gene Expression (1 IFC)	100-6266
Control Line Fluid Kit—192.24	100-4058
192.24 GE Dynamic Array 4X Reagent Kit—10 IFCs	102-0166
Bulk Dilution Reagent—25 mL	100-8726
Reverse Transcription Master Mix	100-6300 (1 tube) 100-6299 (5 tubes)
Preamp Master Mix	100-5580 (1 tube) 100-5581 (5 tubes)

3 Research Applications

Do you have products that support NGS applications for viral RNA?

Fluidigm offers two products that support NGS library prep: Advanta™ NGS Library Prep chemistry for generating targeted NGS libraries, and the Advanta RNA-Seq NGS Library Prep Kit for generating full-length stranded RNA-seq libraries. Both are designed for use on Illumina® sequencers.

Can viral RNA be captured by the Advanta RNA-Seq NGS Library Prep Kit?

Yes, granted the target is a polyadenylated RNA virus, such as SARS-CoV-2.

Can I develop custom panels for SARS-CoV-2 or other respiratory pathogen research?

The Fluidigm D3™ Assay Design Group can assist in creating custom assay panels for targeted NGS library prep, gene expression and SNP genotyping. Specific genomes of interest can be uploaded into a user's D3 account for design, as well. Customers can request a design by emailing or contacting their field application scientist (FAS).

Learn more about COVID-19 and Fluidigm innovation at fluidigm.com/covid-19

Learn more about the Biomark at fluidigm.com/biomark

For convenience, we have created the following bundles for ordering:

- IFC and reagent kit bundles: These bundles include all necessary components for running the IDT assay on the 192.24 Dynamic Array IFC for Gene Expression.
 - 192.24 GE IFC & 4X Reagent Kit—10 IFCs (PN 102-0167)
 - 192.24 GE IFC & 4X Reagent Kit—50 IFCs (PN 102-0168)

Why is a new 4X Assay Loading Reagent (ALR) in the protocol?

The higher concentration of ALR (PN 102-0135) ensures compatibility with the IDT primer/probe set.

Has Fluidigm developed pre-designed panels for SARS-CoV-2 research?

The D3 Assay Design Group has designed primers for two applications based on the NCBI genome build RefSeq NC_045512.2. These primers have been tested *in silico* to be specific to the SARS-CoV-2 genome and are intended for research use only:

- Targeted DNA Seq Library Primers for use with the Advanta NGS Library Prep Kit
 - A full SARS-CoV-2 design covering >98% of the genome. Panel consists of amplicons ranging from 150 to 180 bp in length.
 - Target regions specific to the S, M, E and N genes are identified.
- Delta Gene™ assays for gene expression
 - Pre-designed assays that target the S, M, E and N genes of SARS-CoV-2

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