Frequently asked questions (FAQs)

TaqPath COVID-19 CE-IVD RT-PCR Kit

Applied Biosystems™ TaqPath™ COVID-19 CE-IVD RT-PCR Kit (Cat. No. A48067) and workflow components.

1. **What instruments, consumables, and reagents do I need for the SARS-CoV-2 RNA detection test? Where can I find the full list of CE-IVD products and protocols?**

   Go to **thermofisher.com/COVID19CEIVD** to see the workflow outlining the instruments, consumables, and reagents required to run a SARS-CoV-2 RNA detection experiment.

2. **What instruments are CE-IVD approved?**

   The TaqPath COVID-19 CE-IVD RT-PCR Kit is currently approved for use with all Applied Biosystems™ 7500 systems (Fast, Fast Dx, and standard), the QuantStudio™ 5 Real-Time PCR Systems (96-well 0.1 mL and 0.2 mL), the QuantStudio™ 5 Dx Real-Time PCR System, and the QuantStudio™ 7 Flex Real-Time PCR System (384-well block).

3. **What specimen types can be tested with the SARS-CoV-2 RNA detection test?**

   The TaqPath COVID-19 CE-IVD RT-PCR Kit is currently approved for use with viral RNA extracted from nasopharyngeal and oropharyngeal swabs, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL). Kit performance was established sing these specimen types. Nasal swabs and mid-turbinate swabs are considered acceptable; however, performance with these specimen types has not been established.

4. **What comes in the TaqPath COVID-19 CE-IVD RT-PCR Kit? Is the master mix included, or does it need to be purchased separately?**

   The TaqPath COVID-19 CE-IVD RT-PCR Kit contains:

   - Applied Biosystems™ TaqPath™ COVID-19 RT-PCR Kit, 1,000 reactions (includes the multiplex assay and an MS2 phage internal positive control (IPC))
   - Applied Biosystems™ TaqPath™ COVID-19 Control (positive control for SARS-CoV-2)
   - Applied Biosystems™ TaqPath™ COVID-19 Control Dilution Buffer
   - Applied Biosystems™ TaqPath™ 1-Step Multiplex Master Mix (No ROX)
   - Package insert—provides the instructions and the link to download the Instructions for Use

   This kit is required for use with a range of validated Applied Biosystems™ real-time PCR instruments.

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5. **What is the sensitivity and specificity of the TaqPath COVID-19 CE-IVD RT-PCR Kit?**

   You can find details of the performance characteristics used to assess analytical performance and clinical performance of the TaqPath COVID-19 CE-IVD RT-PCR Kit in the CE-IVD version of the **Instructions for Use (IFU)**. These include: limit of detection (10 genomic copy equivalents (GCE)/reaction), reactivity (homology of assay designs to known SARS-CoV-2 genomes), interfering substances analysis (no false-positive results were observed for any of the substances and concentrations tested), cross-reactivity (*in silico* analysis of 43 organisms for potential cross-reactivity or interference), and clinical evaluation of 60 contrived positive and 60 negative specimens to evaluate kit performance. These characteristics taken together help to confirm that the test performs as expected.

6. **Can you provide any details on how Thermo Fisher Scientific completed their validation and limit of detection (LOD) studies?**

   Viral RNA and MS2 were added to each sample after the addition of the Invitrogen™ Proteinase K, magnetic beads, and binding solution. GCE/mL and GCE/reaction calculations were based on the 400 µL specimen input volume, 50 µL elution volume, and 5 µL template input volume (assuming 100% extraction efficiency). For our 1X LOD, we spiked 100 copies of the RNA into the prep (100 copies/400 µL) for a theoretical RNA concentration of 2 copies/µL in the eluate. Final (theoretical) concentration in the reaction = 5 µL x 2 copies/µL = 10 copies/reaction. Additional details can be found in the **Instructions for Use** under "Performance characteristics".

7. **What genes are targeted, and why is it important to have 3 targets in the assay?**

   The TaqPath COVID-19 CE-IVD RT-PCR Kit targets regions of three coronavirus genes: *orf-1ab*, the gene for the S protein, and the gene for the N protein. These target areas are specific to the SARS-CoV-2 virus, reducing the risk of detecting other coronaviruses. They also exhibit lower mutation entropy. Lastly, our assay continues to maintain targeted specificity to 100% of currently available complete genomes of SARS-CoV-2.

8. **Does each individual component have its own expiration date, or does the entire kit have the same expiration date?**

   Each component has an expiration date, and each outer box is labeled with an expiration date. PCR reagents have a date 12 months from manufacturing, while the control and control buffer have 9 months of shelf life from the date of manufacture, so the shelf life for the entire kit is 9 months.

9. **Will the SARS-CoV-2 positive control work with assays from other manufacturers?**

   Our positive control sequence is designed specifically for the target regions in our assay only. We have not tested our control with assays from other manufacturers.

10. **Is the MS2 phage control infectious? Can you share the concentration of the MS2 phage?**

    The MS2 control is a critical internal control for the test, and although it is not infectious to humans, laboratories should always employ universal precautions in the handling of all samples and reagents. The MS2 phage is active and can infect its hosts; hosts are *Escherichia coli* and other members of the Enterobacteriaceae. The MS2 concentration is proprietary.

11. **What if I switch out a component or step of the CE-IVD workflow?**

    We cannot support the use of any products that are not listed in the protocol. This includes any workflow changes (automation, liquid handling, etc.). Any deviation from the CE-IVD protocol is considered off-label use and invalidates compliance with the IFU.

12. **Why is RNaseP not used in your TaqPath COVID-19 CE-IVD RT-PCR Kit?**

    By design, our kit does not have a sample adequacy control (SAC). That is usually an assay for a human target (i.e., RNaseP). We see a high degree of variation with the 16S RNaseP assay, which makes its use it as a SAC unreliable. Detection of the RNaseP control is dependent on a minimum number of human cells being present in the sample. RNaseP mRNA and viral RNA will have different stability properties in the collected sample (with or without storage prior to analysis). Considering this difference, the detection of RNaseP is not an accurate sample collection control—a sample correctly collected and processed may be negative for RNaseP simply because the mRNA was less stable than the viral RNA. A similar RNA virus control (e.g., MS2 phage) is more relevant as a control in the extraction and amplification steps.

13. **Can I make a change to the protocol for the TaqPath COVID-19 CE-IVD RT-PCR Kit?**

    Any changes to the protocol are considered off-label use and invalidate compliance with the IFU.

14. **What is the difference between the original Applied Biosystems™ MagMAX™ Viral/Pathogen kit (Cat. No. A42352, A48310) and the MagMAX™ Viral/Pathogen II kit (Cat. No. A48383)?**

    There are two key differences:

    - A proprietary raw material in the bead component. The chemistry driving the RNA to the beads is identical, and the nucleic acid binding capacity provides similar or equivalent results between the two products.
    - The MagMAX Viral/Pathogen II kit (Cat. No. A48383) is only available in bulk format; there is no 100-reaction option for this kit.

15. **Is the MagMAX Viral/Pathogen II kit compatible with the TaqPath COVID-19 CE-IVD RT-PCR Kit?**

    Yes. You can use the MagMAX Viral/Pathogen II kit just as you would use the MagMAX Viral/Pathogen kit with the TaqPath COVID-19 CE-IVD RT-PCR Kit. When using the 200 µL sample input protocol, please note that the amount of eluate used for the PCR will be changed to 10 µL (5 µL eluate used for PCR when the original sample input is 400 µL).

16. **How is efficiency improved with the MagMAX Viral/Pathogen II kit?**

    The MagMAX Viral/Pathogen II kit protocol is faster and more flexible. It reduces the number or wash steps from 3 to 2, and the sample input from 400 µL to 200 µL, allowing twice as many samples to be purified and processed, resulting in more tests.

17. **In the COVID-19 Interpretive Software, are there specific plate positions that must be used for positive and negative controls? Will the interpretive software consider replicates? If not, what would you recommend in case of a discrepancy?**

    Controls can be placed anywhere on the plate. They need to be assigned and defined correctly for the interpretive software, which can't process replicate samples. If a customer chooses to run replicate samples, the wells must be labeled differently (e.g., Sample 1a and Sample 1b, instead of two wells labeled "Sample 1").

18. **How is the report generated for the TaqPath COVID-19 CE-IVD RT-PCR kit?**

    The report is generated by the COVID-19 interpretive software. It will:

    - Automatically interpret genetic analysis results from the TaqPath COVID-19 CE-IVD RT-PCR Kit
    - Perform a QC check against all controls on the plate following instrument data analysis
    - Generate a report for each specimen

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19. How can I obtain software for the TaqPath COVID-19 CE-IVD RT-PCR Kit?

To obtain the software, contact your local support team or look up the local service phone number on the website (see below).

Go to **thermofisher.com/contactus**

- In the Step One pane, click Instrument Service.
- In the Step Two pane, enter the name of your real-time PCR instrument, then select your location from the drop-down list.
- To obtain the software, call the local service phone number that is displayed on the screen.

20. Can I use synthetic RNA from another company with your TaqPath COVID-19 CE-IVD RT-PCR Kit?

When following the CE-IVD Instructions for Use, you must use the TaqPath COVID-19 Positive Control as the positive control with the kit.

21. When using the TaqPath COVID-19 CE-IVD RT-PCR Kit, can you install the interpretive software on the instrument computer?

No, the interpretive software must be installed on a separate computer that meets the specifications in the Quick Reference Guide.

22. When using the TaqPath COVID-19 CE-IVD RT-PCR Kit, how important is it to vortex the reaction plate?

It is extremely important to thoroughly mix and spin down the RT-PCR reaction plate. The TaqPath 1-Step Multiplex Master Mix (No ROX) is a 4X master mix and is extremely viscous. If the plate is not mixed sufficiently, this can cause optical mixing in the early cycles of the qPCR. Optical mixing can lead to RT-PCR baseline instability, resulting in the QC failure of entire plates.

23. What sample collection swabs would you recommend for same-day transport and processing?

The Instructions for Use (IFU) only specify specimen types—upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL). The language in our product IFU was developed with the support of the FDA to enable customers to have flexibility in the choice of storage components utilized. This means that the choice of one storage matrix over another does not impact compliance with the product IFU. This allows labs to have flexibility based on availability of storage media and vials that conform with information posted on the FDA website. It is important to note that samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences. We did not validate any specific transport and storage solution; your lab is responsible for choosing and verifying the effectiveness of the transport media you have selected, and complying with manufacturer guidance for the storage media and vial you intend to use. You can find further guidance on alternatives to viral transport medium (VTM) or universal transport medium (UTM) that the FDA deems appropriate, on their website.

24. Can you provide any resources for laboratory information systems (LIMS) integration?

Please see the LIMS integration technical information package **here**.

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CE IVD For *In Vitro* Diagnostic Use.