Applied Biosystems™ TaqPath™ COVID-19 CE-IVD RT-PCR Kit

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Field Application Scientist

For in vitro diagnostic use.
TaqPath COVID-19 CE-IVD RT-PCR Kit

- Multiplex reverse transcriptase Real-Time PCR Kit for detection of SARS-CoV-2 RNA

- Compatible Instruments:
  - Applied Biosystems 7500
  - Applied Biosystems 7500 Fast
  - Applied Biosystems 7500 Fast Dx
  - *QuantStudio 5 Real-Time PCR System
    - 0.2ml variant

* Coming soon
Features of the TaqPath COVID-19 CE-IVD RT-PCR Kit

Our highly-sensitive, real-time PCR solution enables your laboratory to run up to 94 specimens on a single plate in less than 2 hours.

- A complete analysis to reporting solution for up to 94 specimens and genetic analysis using real-time PCR to diagnostic report generation in less than 2 hours.

- In silico analysis suggest 100% coverage of available complete genomes for SARS-CoV-2 covered (April 10th 2020)

- Assay targets ORF-1ab, spike (S) protein and nucleocapsid (N) protein regions were chosen with the goal to have high specificity and lower risk for mutation

- COVID-19 Interpretive Software, which automatically converts genetic analysis data into reporting, to reduce interpretation errors
Our TaqPath COVID-19 CE-IVD RT-PCR Kit targets areas specific to the SARS-CoV-2 virus, reducing the risk of detecting other coronaviruses.

- Applied Biosystems TaqPath COVID-19 assay targets areas specific to the SARS-CoV-2 virus, reducing the risk of detecting other coronaviruses.

- Our assay targets regions of the SARS-CoV-2 virus outside of regions that are known for being mutation regions to reduce risk of loss of specificity.

- Our assay covers 100% of available SARS-CoV-2 sequences available on April 10th, 2020.
Sample-to-Answer Workflow for TaqPath™ COVID-19 CE-IVD RT-PCR Kit

**Sample collection**

**Sample Preparation**

*Recommended product:*
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, MagMAX™-96 Viral RNA Isolation Kit, or equivalent (Manual or automated processing with KingFisher™ System)

**Plate Setup**

**Analysis and Reporting**

*Required as part of the TaqPath COVID-19 CE-IVD Kit Workflow

**Perform RT-PCR**

*Coming soon

**Transfer Data**

Applied Biosystems™
COVID-19 Interpretive Software

Applied Biosystems™
COVID-19 SDS or EDS run file

Applied Biosystems™
7500 Fast Dx Real-Time PCR System or 7500 Fast Real-Time PCR System

7500 Real-Time PCR System

***QuantStudio5 Real-Time PCR System

Please Note: Authorized laboratories using the TaqPath COVID-19 CE-IVD Kit will perform the TaqPath COVID-19 CE-IVD Kit as outlined in the Instructions for Use.

*Recommended as part of the TaqPath COVID-19 CE-IVD Kit Workflow

**Required as part of the TaqPath COVID-19 CE-IVD Kit Workflow

***Coming soon
Three Approved Sample Types

Approved specimen types* include:
- nasopharyngeal swabs,
- nasopharyngeal aspirate (nasal aspirate), and
- bronchoalveolar lavage (BAL)

*Further sample types undergoing testing and validation
Guidelines for RNA extraction

• The quality of the sample preparation (purified RNA) may influence the quality of the qPCR test

• Laboratories shall only use the purification method they have selected

• Laboratories that do not have any selected method, may use the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, MagMAX™ Viral/Pathogen II, MagMAX™-96 Viral RNA Isolation Kit, or equivalent
Extraction Workflow

- Prepare Work Area ~ 5 min
- Prepare 80% ETOH ~ 5 min
- Prepare Processing Plates ~ 5 min
- Sample Preparation ~ 20 min
- Sample Extraction on KingFisher System or Manual ~ 25 min
- Sample Storage
Extraction

- TaqPath COVID-19 CE-IVD RT-PCR Kit has been tested and found compatible with many sample extraction methods
  - Subject to internal Laboratory testing

- Provide highly pure viral RNA

- Free from inhibitors

- Nucleic Acid integrity maintained
Guidelines for RNA extraction

• MS2 Phage Control provided in the kit must be used to verify the efficacy of the sample preparation and the absence of inhibitors in the RT-PCR reaction

• Use Nuclease-free Water (not DEPC-Treated) containing 10 μL MS2 Phage Control as the Negative Control.
  • The purified Negative Control is used as the Negative Control for RT-PCR.

• Add 10 μL of MS2 Phage Control to each sample well and to the Negative Control well just before lysis during RNA extraction.
Sample-to-Answer Workflow for TaqPath™ COVID-19 CE-IVD RT-PCR Kit

**Sample collection** → **Sample Preparation** → **Plate Setup** → **Perform RT-PCR** → **Analysis and Reporting** → **Transfer Data**

- **COVID-19**
- **Same Day Turnaround Time**
- **Service and support available throughout the entire process**

**Recommended product:**
- MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit,
- MagMAX Viral RNA Nucleic Acid Isolation Kit or equivalent

(Mandatory or automated processing with KingFisher™ System)

**Please Note:** Authorized laboratories using the TaqPath COVID-19 CE-IVD Kit will perform the TaqPath COVID-19 CE-IVD Kit as outlined in the Instructions for Use.

*Recommended as part of the TaqPath COVID-19 CE-IVD Kit Workflow

**Required** as part of the TaqPath COVID-19 CE-IVD Kit Workflow

***Coming Soon***

**Applied Biosystems™**
- COVID-19 Interpretive Software
- 7500 Real-Time PCR System
- QuantStudio5 Real-Time PCR System

**MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit**
- Manual or automated processing with KingFisher™ System

**Analysis and Reporting**
- SDS or EDS run file

**Applied Biosystems™**
- 7500 Fast Dx Real-Time PCR System
- 7500 Fast Real-Time PCR System
- 7500 Real-Time PCR System

**SDS or EDS run file**

~115 min
### What’s in the Kit?

#### TaqPath COVID-19 CE-IVD RT-PCR Kit, 1000 Reactions (Cat. No. A48067)

<table>
<thead>
<tr>
<th>Included in CE-IVD RT PCR</th>
<th>Description</th>
<th>Contains</th>
</tr>
</thead>
<tbody>
<tr>
<td>TaqPath COVID-19 CE-IVD RT PCR Kit, 1000 reactions</td>
<td>COVID-19 Real-time PCR Assay Multiplex (Gene Orf-1ab, N Protein, S Protein, MS2)</td>
<td>1 tube (1,500µl)</td>
</tr>
<tr>
<td></td>
<td>MS2 Phage Control</td>
<td>10 tubes (1ml)</td>
</tr>
<tr>
<td></td>
<td>TaqPath COVID-19 Control IVT RNA Control</td>
<td>10 tubes (10µl (spec: 10^4 copies/µL))</td>
</tr>
<tr>
<td></td>
<td>TaqPath COVID-19 Control Dilution Buffer for RNA Control</td>
<td>10 tubes (250µl)</td>
</tr>
<tr>
<td></td>
<td>TaqPath™ 1-Step Multiplex Master Mix, (No ROX)</td>
<td>1 bottle (10 ml)</td>
</tr>
</tbody>
</table>

#### Gene | Dye | Quencher
---|---|---
ORF1ab | FAM | QSY
N Protein | VIC | QSY
S Protein | ABY | QSY
MS2 (Internal Positive Control) | JUN | QSY

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For in vitro diagnostic use.
In summary, a highly specific multiplex assay for SARS-CoV-2 pathogen detection

As viruses mutate and spread, specificity to both the current virus and future mutations is absolutely necessary. Our multiplex assay provides laboratories with this certainty, and more…

• Highly sensitive and specific assay utilizing TaqMan technology, considered the gold standard for PCR.
• In silico analysis suggest 100% coverage of available complete genomes for SARS-CoV-2 covered (April 10th 2020)
• Our assay targets regions of the SARS-CoV-2 virus outside of regions that are known for being mutation regions to reduce risk of loss of specificity
• Multiplex kit available in 1000 reactions per kit to help maximize specimen throughput

Visit thermofisher.com/COVID19CEIVD for full intended use statement
Experiment Setup using TaqPath™ 1-Step Multiplex Master Mix (No ROX™)

Sample Reaction Setup

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per Sample or Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)</td>
<td>6.25 µL</td>
</tr>
<tr>
<td>COVID-19 Real Time PCR Assay Multiplex</td>
<td>1.25 µL</td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>12.50 µL</td>
</tr>
<tr>
<td>Total Reaction Mix volume</td>
<td>20.0 µL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per reaction</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sample reaction</td>
</tr>
<tr>
<td>Reaction Mix</td>
<td>20.0 µL</td>
</tr>
<tr>
<td>Purified Sample nucleic acid</td>
<td>5.0 µL</td>
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<tr>
<td>Positive Control (TaqPath™ COVID-19 Control)</td>
<td>—</td>
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<tr>
<td>Nuclease-free Water</td>
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<tr>
<td>Purified Negative Control</td>
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<tr>
<td>Total volume</td>
<td>25.0 µL</td>
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</tbody>
</table>

Real-Time PCR Instrument Thermal Protocol

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Number of cycles</th>
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</thead>
<tbody>
<tr>
<td>UNG incubation</td>
<td>25°C</td>
<td>2 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Reverse transcription</td>
<td>50°C</td>
<td>10 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Activation</td>
<td>96°C</td>
<td>2 minutes</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>96°C</td>
<td>3 seconds</td>
<td>40</td>
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<tr>
<td>Annel / extension</td>
<td>60°C</td>
<td>30 seconds</td>
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</tbody>
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Example Plate Layout

Each Reaction
- ORF1ab (FAM)
- N gene (VIC)
- S gene (640)
- MS2 (BHQ)

Example Plate Layout

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
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</table>

ThermoFisher Scientific
COVID-19 Interpretive Software

For in vitro diagnostic use.
The New **Applied Biosystems COVID-19 Interpretive Software** helps your lab decrease analysis and interpretation time, and eliminate subjective interpretation.

- Automatically interpret the data results from the TaqPath COVID-19 CE-IVD RT-PCR genetic analysis.

- Following Real-Time PCR run, the COVID19 Interpretive Software performs a QC check against all controls on the plate and alert user.

- Software generates interpretative report for each specimen.

- **COVID-19 Interpretive Software helps reduce time to diagnosis, along with risk of user error in translation or interpretation.**
# Software and Analysis Recommendations

<table>
<thead>
<tr>
<th>Instruments</th>
<th>Instrument Software</th>
<th>Data Analysis Software</th>
</tr>
</thead>
</table>
| **Applied Biosystems™ 7500 Fast Dx or 7500 Fast or 7500 Real-Time PCR Instrument** | For 7500 Fast Dx use Software v1.4.1  
For 7500 Fast use Software v2.3 or v1.5.1  
For 7500 use Software v2.3 or v1.5.1 | COVID-19 Interpretive Software v1.2 |
| **Applied Biosystems™ QuantStudio5 Real-time PCR Instrument** | QuantStudio Design and Analysis Software FW v1.3.3 + DA v1.5.1 | coming soon |

<table>
<thead>
<tr>
<th>ORF1ab</th>
<th>N gene</th>
<th>S gene</th>
<th>MS2</th>
<th>Status</th>
<th>Result</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>Invalid</td>
<td>N/A</td>
<td>Repeat test. If the repeat result remains invalid, consider collecting a new specimen.</td>
</tr>
<tr>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>POS</td>
<td>Valid</td>
<td>SARS-CoV-2 Not Detected</td>
<td>Report results to healthcare provider. Consider testing for other viruses</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Only one SARS-CoV-2 target = POS</td>
<td>POS or NEG</td>
<td>Valid</td>
<td>SARS-CoV-2 Inconclusive</td>
<td>Repeat test. If the repeat result remains inconclusive, contact CDC immediately for instructions for transfer of the specimen to CDC for additional testing and guidance.</td>
<td></td>
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</tr>
<tr>
<td>Two or more SARS-CoV-2 targets = POS</td>
<td>POS or NEG</td>
<td>Valid</td>
<td>SARS-CoV-2 Detected</td>
<td>Report results to healthcare provider and CDC. Contact CDC immediately for instructions for transfer of the specimen to CDC for additional testing and guidance.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The Applied Biosystems COVID-19 Interpretive Software helps you to **decrease analysis and interpretation time and reduces risk of user interpretation error.**
COVID-19 Interpretive Software Features & Benefits

The Applied Biosystems COVID-19 Interpretive Software makes it easier for you to adopt our solution into your current environment.

* Purchaser is responsible for any LIMS integration implementation.
Reagents required – not supplied

- For a list of required reagents not supplied, see instructions for use below


<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory freezer</td>
<td>-20°C to -10°C</td>
</tr>
<tr>
<td>Centrifuge, with a rotor for microplates</td>
<td>MLS</td>
</tr>
<tr>
<td>Microcentrifuge</td>
<td>MLS</td>
</tr>
<tr>
<td>Laboratory mixer, Vortex or equivalent</td>
<td>MLS</td>
</tr>
<tr>
<td>Single and multi-channel adjustable pipets (1.00 µL to 1,000.0 µL)</td>
<td>MLS</td>
</tr>
<tr>
<td>Cold block or ice</td>
<td>MLS</td>
</tr>
<tr>
<td>Nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)</td>
<td>thermofisher.com/plastics</td>
</tr>
<tr>
<td>Sterilize aerosol carrier (filtered) pipette tips</td>
<td>thermofisher.com/pipettips</td>
</tr>
</tbody>
</table>

Contact us for specific part numbers, as these may vary depending on instrument model.
Performance Data: COVID-19 CE-IVD RT-PCR Kit
Limit of Detection (LoD) & Reactivity (Inclusivity)

The LoD study established the lowest SARS-CoV-2 viral concentration (Genomic Copy Equivalents of GCE) that can be detected by the TaqPath™ COVID-19 CE-IVD RT-PCR Kit in a particular specimen type at least 95% of the time. Banked Nasopharyngeal swab (NP) and Bronchoalveolar lavage (BAL) samples, obtained from U.S. patients in the years 2015-2019, were pooled, respectively, and spiked with purified SARS-CoV-2 RNA at several concentrations and processed through the TaqPath COVID-19 CE IVD RT PCR Kit workflow. A three phase approach was used to determine the LoD for each specimen type.

**Table 4  LoD results**

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Limit of Detection (GCE/mL)</th>
<th>Limit of Detection (GCE/reaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchoalveolar lavage</td>
<td>250 GCE/mL</td>
<td>10 GCE/reaction</td>
</tr>
<tr>
<td>Nasopharyngeal swab</td>
<td>250 GCE/mL</td>
<td>10 GCE/reaction</td>
</tr>
</tbody>
</table>

**Reactivity (Inclusivity)**

The assays were mapped to 185 complete SARS-CoV-2 genomes of human host in GenBank and GiSAID databases as of March 5, 2020. Primer and probes sequences for SARS-CoV-2 ORF1ab, S gene, and N gene assays had 100% homology to all SARS-CoV-2 isolates analyzed, with one exception. EPI_ISL_407084 showed a mismatch at position 7 from the 5’ end of the reverse primer (23 nt length) corresponding to 95.6% homology. The mismatch is located at the 5’end of the primer and does not affect the test performance.
A clinical evaluation study was performed to evaluate the performance of the TaqPath RT-PCR COVID-19 Kit using nasopharyngeal swab (NP) and bronchoalveolar lavage (BAL) specimens.

A total of sixty (60) contrived positive specimens were tested:
- 30 contrived positive nasopharyngeal swab (NP) specimens
- 30 contrived positive bronchoalveolar lavage (BAL) specimens

Samples were contrived by spiking known concentrations of isolated SARS-CoV-2 RNA, relative to the product LoD, into matrices which were determined to be negative by the TaqPath RT-PCR COVID-19 Kit prior to spiking in the RNA.

In addition to the contrived positive specimen, sixty (60) negative specimens were tested:
- 30 negative nasopharyngeal swab (NP) specimens
- 30 negative samples bronchoalveolar lavage (BAL) specimens
Clinical evaluation study outcome

### Table 6  BAL Clinical Evaluation Study

<table>
<thead>
<tr>
<th>Final RNA Concentration in Sample</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X LoD</td>
<td>5/5  positive or detected</td>
</tr>
<tr>
<td>3X LoD</td>
<td>5/5  positive or detected</td>
</tr>
<tr>
<td>2X LoD</td>
<td>20/20  positive or detected</td>
</tr>
<tr>
<td>Negative</td>
<td>30/30  not detected</td>
</tr>
</tbody>
</table>

### Table 7  NP Clinical Evaluation Study

<table>
<thead>
<tr>
<th>Final RNA Concentration in Sample</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X LoD</td>
<td>5/5  positive or detected</td>
</tr>
<tr>
<td>3X LoD</td>
<td>5/5  positive or detected</td>
</tr>
<tr>
<td>2X LoD</td>
<td>20/20  positive or detected</td>
</tr>
<tr>
<td>Negative</td>
<td>30/30  not detected</td>
</tr>
</tbody>
</table>
We would love to hear from you!
• Further SARS-CoV-2 products:

• TaqPath COVID-19 CE-IVD RT-PCR webpage:

• qPCR Learning Centre:
Our Africa Network & Contact Details

SA Headquarter for Africa
6 sites in SA, Offices in Kenya, Morocco and Ivory Coast

250+
Employees in Africa

50+
Engineers & Applications Specialists

If you would like any further information, please let us know!
Contact AfricaLeads@thermofisher.com
• Joanne Mizrachi – Sales Queries
  • joanne.bradfield@thermofisher.com
• Riegardt Johnson – Technical Queries
  • riegardt.johnson@thermofisher.com