



## Assay Profile

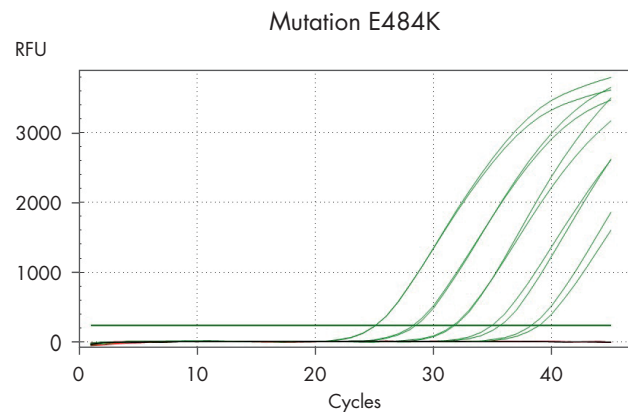
# LNA-based qPCR assays for the genotyping of SARS-CoV-2 variants of concern (VOCs)

A robust design pipeline with several parameters for optimal primer-probe combinations was wet-lab tested for the mutations. The specificity and sensitivity of the assays were validated using the QIAprep&® Viral RNA UM Kit. The user should order primers and probes from a partnering oligo provider. QIAGEN can provide recommendations (in this case, our trusted partner [biomers.net](https://www.biomers.net)), but it is up to the end-user to validate the assays in their workflow. Assay validation should be carried out to ensure that the laboratory workflow performs adequately for the circulating viruses.



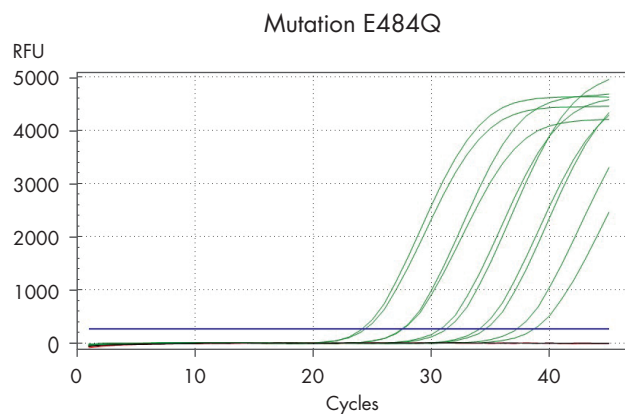
| IVT copies/<br>reaction | Bio-Rad CFX | ABI<br>QuantStudio | QIAGEN<br>Rotor-Gene Q |
|-------------------------|-------------|--------------------|------------------------|
|                         | Ct          |                    |                        |
| 10 <sup>5</sup>         | 23.4        | 23.6               | 23.4                   |
| 10 <sup>4</sup>         | 26.7        | 27.4               | 27.0                   |
| 10 <sup>3</sup>         | 30.0        | 30.6               | 30.1                   |
| 10 <sup>2</sup>         | 33.2        | 34.4               | 33.1                   |
| 10 <sup>1</sup>         | 37.4        | 37.7               | 36.4                   |

**Specific and sensitive detection of the mutation T478K.** In green: amplification curves from dilution series (10<sup>5</sup>–10<sup>1</sup> cp/rxn, in duplicates) of an in-vitro transcript (IVT) bearing the SARS-CoV-2 mutation T478K. In red: amplification curve of an IVT of the corresponding WT sequence from SARS-CoV-2 at 10<sup>7</sup> cp/rxn. In black (overlapping with the red line) is the NTC.



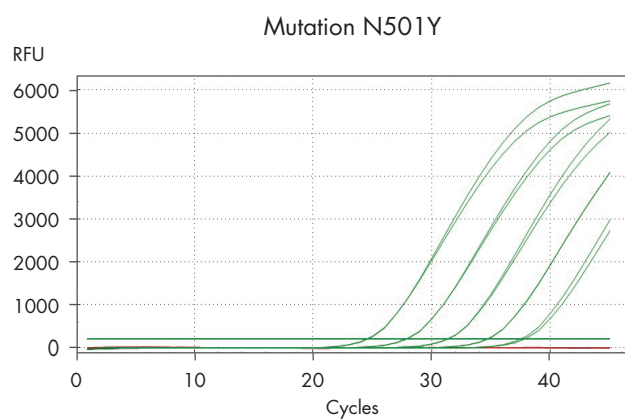
| IVT copies/<br>reaction | Bio-Rad CFX | ABI<br>QuantStudio | QIAGEN<br>Rotor-Gene Q |
|-------------------------|-------------|--------------------|------------------------|
|                         | Ct          |                    |                        |
| 10 <sup>5</sup>         | 25.1        | 25.6               | 25.1                   |
| 10 <sup>4</sup>         | 28.3        | 29.3               | 28.5                   |
| 10 <sup>3</sup>         | 31.6        | 33.4               | 32.0                   |
| 10 <sup>2</sup>         | 35.2        | 36.7               | 35.6                   |
| 10 <sup>1</sup>         | 38.5        | 41.3               | 39.1                   |

**Specific and sensitive detection of the mutation E484K.** In green: amplification curves from dilution series (10<sup>5</sup>–10<sup>1</sup> cp/rxn, in duplicates) of an in-vitro transcript (IVT) bearing the SARS-CoV-2 mutation E484K. In red: amplification curve of an IVT of the corresponding WT sequence from SARS-CoV-2 at 10<sup>7</sup> cp/rxn. In black (overlapping with the red line) is the NTC.



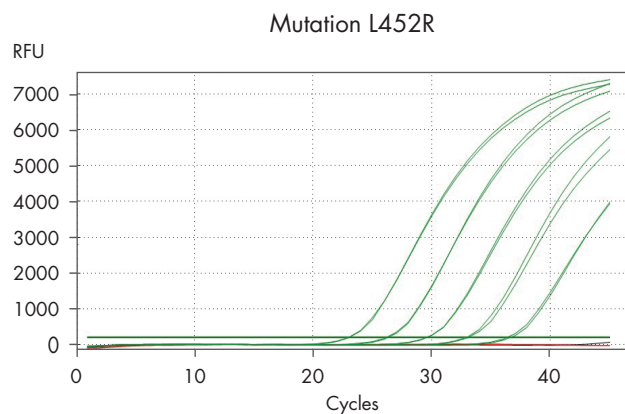
| IVT copies/<br>reaction | Bio-Rad CFX | ABI<br>QuantStudio | QIAGEN<br>Rotor-Gene Q |
|-------------------------|-------------|--------------------|------------------------|
|                         | Ct          |                    |                        |
| 10 <sup>5</sup>         | 24.3        | 24.4               | 24.2                   |
| 10 <sup>4</sup>         | 27.6        | 27.8               | 27.6                   |
| 10 <sup>3</sup>         | 31.0        | 31.1               | 30.9                   |
| 10 <sup>2</sup>         | 34.3        | 34.8               | 34.1                   |
| 10 <sup>1</sup>         | 38.0        | 39.3               | 38.1                   |

**Specific and sensitive detection of the mutation E484Q.** In green: amplification curves from dilution series ( $10^5$ – $10^1$  cp/rxn, in duplicates) of an in-vitro transcript (IVT) bearing the SARS-CoV-2 mutation E484Q. In red: amplification curve of an IVT of the corresponding WT sequence from SARS-CoV-2 at  $10^7$  cp/rxn. In black (overlapping with the red line) is the NTC.



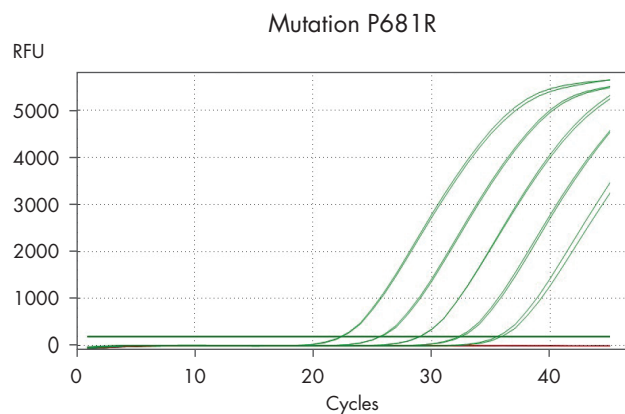
| IVT copies/<br>reaction | Bio-Rad CFX | ABI<br>QuantStudio | QIAGEN<br>Rotor-Gene Q |
|-------------------------|-------------|--------------------|------------------------|
|                         | Ct          |                    |                        |
| 10 <sup>5</sup>         | 24.5        | 24.4               | 24.12                  |
| 10 <sup>4</sup>         | 27.8        | 27.8               | 27.25                  |
| 10 <sup>3</sup>         | 31.2        | 31.1               | 31.01                  |
| 10 <sup>2</sup>         | 34.7        | 34.4               | 33.99                  |
| 10 <sup>1</sup>         | 37.6        | 37.6               | 38.21                  |

**Specific and sensitive detection of the mutation N501Y.** In green: amplification curves from dilution series ( $10^5$ – $10^1$  cp/rxn, in duplicates) of an in-vitro transcript (IVT) bearing the SARS-CoV-2 mutation N501Y. In red: amplification curve of an IVT of the corresponding WT sequence from SARS-CoV-2 at  $10^7$  cp/rxn. In black (overlapping with the red line) is the NTC.



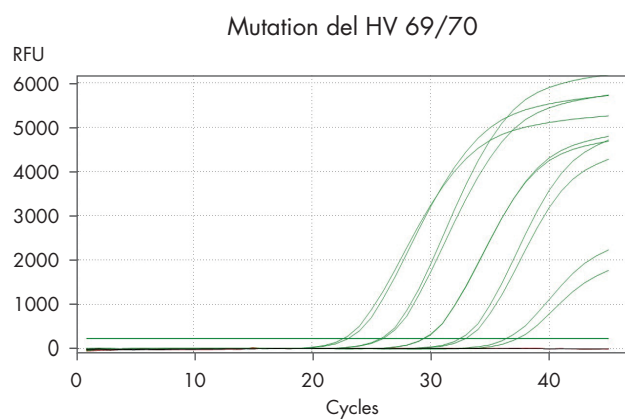
| IVT copies/<br>reaction | Bio-Rad CFX | ABI<br>QuantStudio | QIAGEN<br>Rotor-Gene Q |
|-------------------------|-------------|--------------------|------------------------|
|                         | Ct          |                    |                        |
| 10 <sup>5</sup>         | 22.9        | 22.6               | 22.72                  |
| 10 <sup>4</sup>         | 26.2        | 25.8               | 25.28                  |
| 10 <sup>3</sup>         | 29.5        | 29.1               | 28.98                  |
| 10 <sup>2</sup>         | 33.0        | 32.4               | 32.65                  |
| 10 <sup>1</sup>         | 36.6        | 35.7               | 35.83                  |

**Specific and sensitive detection of the mutation L452R.** In green: amplification curves from dilution series ( $10^5$ – $10^1$  cp/rxn, in duplicates) of an in-vitro transcript (IVT) bearing the SARS-CoV-2 mutation L452R. In red: amplification curve of an IVT of the corresponding WT sequence from SARS-CoV-2 at  $10^7$  cp/rxn. In black (overlapping with the red line) is the NTC.



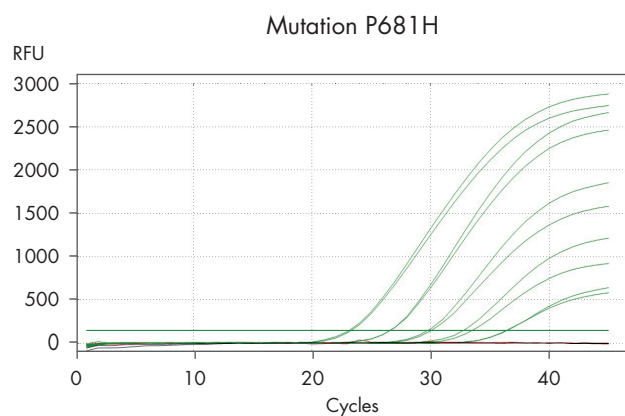
| IVT copies/<br>reaction | Bio-Rad CFX | ABI<br>QuantStudio | QIAGEN<br>Rotor-Gene Q |
|-------------------------|-------------|--------------------|------------------------|
|                         | Ct          |                    |                        |
| 10 <sup>5</sup>         | 22.4        | 22.30              | 23.0                   |
| 10 <sup>4</sup>         | 25.7        | 25.63              | 25.9                   |
| 10 <sup>3</sup>         | 29.0        | 29.02              | 29.3                   |
| 10 <sup>2</sup>         | 32.4        | 32.31              | 33.2                   |
| 10 <sup>1</sup>         | 35.4        | 35.50              | 36.1                   |

**Specific and sensitive detection of the mutation P681R.** In green: amplification curves from dilution series (10<sup>5</sup>–10<sup>1</sup> cp/rxn, in duplicates) of an in-vitro transcript (IVT) bearing the SARS-CoV-2 mutation P681R. In red: amplification curve of an IVT of the corresponding WT sequence from SARS-CoV-2 at 10<sup>7</sup> cp/rxn. In black (overlapping with the red line) is the NTC.



| IVT copies/<br>reaction | Bio-Rad CFX | ABI<br>QuantStudio | QIAGEN<br>Rotor-Gene Q |
|-------------------------|-------------|--------------------|------------------------|
|                         | Ct          |                    |                        |
| 10 <sup>5</sup>         | 21.47       | 21.67              | 21.57                  |
| 10 <sup>4</sup>         | 24.80       | 24.65              | 24.82                  |
| 10 <sup>3</sup>         | 27.83       | 28.26              | 27.80                  |
| 10 <sup>2</sup>         | 31.62       | 31.52              | 31.49                  |
| 10 <sup>1</sup>         | 34.24       | 35.64              | 35.04                  |

**Specific and sensitive detection of the deletion mutation HV 69/70.** In green: amplification curves from dilution series (10<sup>5</sup>–10<sup>1</sup> cp/rxn, in duplicates) of an in-vitro transcript (IVT) bearing the SARS-CoV-2 deletion mutation HV 69/70. In red: amplification curve of an IVT of the corresponding WT sequence from SARS-CoV-2 at 10<sup>7</sup> cp/rxn. In black (overlapping with the red line) is the NTC.



| IVT copies/<br>reaction | Bio-Rad CFX | ABI<br>QuantStudio | QIAGEN<br>Rotor-Gene Q |
|-------------------------|-------------|--------------------|------------------------|
|                         | Ct          |                    |                        |
| 10 <sup>5</sup>         | 23.18       | 23.17              | 24.17                  |
| 10 <sup>4</sup>         | 26.37       | 26.54              | 27.13                  |
| 10 <sup>3</sup>         | 29.64       | 29.87              | 29.53                  |
| 10 <sup>2</sup>         | 32.77       | 33.08              | 32.85                  |
| 10 <sup>1</sup>         | 35.70       | 36.34              | 37.46                  |

**Specific and sensitive detection of the mutation P681H.** In green: amplification curves from dilution series (10<sup>5</sup>–10<sup>1</sup> cp/rxn, in duplicates) of an in-vitro transcript (IVT) bearing the SARS-CoV-2 mutation P681H. In red: amplification curve of an IVT of the corresponding WT sequence from SARS-CoV-2 at 10<sup>7</sup> cp/rxn. In black (overlapping with the red line) is the NTC.

**Genotyping assay compatibility with control genes in a duplex reaction**

| Mutation assays | Control genes |    |        |      |
|-----------------|---------------|----|--------|------|
|                 | N1            | N2 | E gene | RdRp |
| N501Y           | ✓             | ✓  | ✓      | ✓    |
| E484K           |               | ✓  | ✓      | ✓    |
| E484Q           |               | ✓  | ✓      | ✓    |
| P681R           | ✓             | ✓  |        |      |
| L452R           | ✓             | ✓  | ✓      | ✓    |
| T478K           |               | ✓  |        |      |
| K417N           | ✓             | ✓  | ✓      | ✓    |
| K417T           |               | ✓  | ✓      |      |
| T20N            | ✓             | ✓  | ✓      |      |
| Del HV 69/70    | ✓             | ✓  | ✓      | ✓    |
| P681H           | ✓             | ✓  | ✓      |      |



To order all your oligos quickly and easily, visit [biomers.net](https://www.biomers.net) at [https://www.biomers.net/en/products/Catalog\\_Products/Primers-for-QIAGEN-SARS-CoV-2-Assays](https://www.biomers.net/en/products/Catalog_Products/Primers-for-QIAGEN-SARS-CoV-2-Assays).



To order the QIAprep&amp; Viral RNA UM Kit, visit QIAGEN at [www.qiagen.com/qiaprepamp-viral-rna-um-kit](http://www.qiagen.com/qiaprepamp-viral-rna-um-kit).



Download a Quick-Start Protocol for the SARS-CoV-2 LNA qPCR Assays at [www.qiagen.com/covid-genotyping-QSP](http://www.qiagen.com/covid-genotyping-QSP).

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The assays described here are intended for molecular biology applications. These assays are not intended for the diagnosis, prevention, or treatment of a disease.

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