

# Impact of the 69-70del mutation in the spike protein of SARS-CoV-2 on the TaqPath COVID-19 Combo Kit

## Background

Recent reports of a deletion mutation in the S gene of the SARS-CoV-2 genome have emerged in the scientific community. The 6-nucleotide deletion (21765–21770) of the S gene results in a deletion of two amino acids at sites 69 (histidine) and (70 valine) in the spike protein commonly referred to as 69-70del. The 69-70del mutation is predominantly observed in B.1.1, B.1.258, and the cluster 5 variant lineages of the SARS-CoV-2. Of these, the B.1.1.7 variant is named by Public Health England as VUI-202012/01 (the first “Variant Under Investigation” in December 2020), which has 17 mutations including 69-70del [1]. While there are several mutations beyond 96-70del observed in other regions of the S gene as well as mutations in other regions of the SARS-CoV-2 genome, such as in the *orf1-ab*, *orf8*, and the N gene, these mutations do not impact our Applied Biosystems™ TaqPath™ COVID-19 Combo Kit.

Viruses constantly change through mutations leading to the emergence of new variants. We regularly monitor post-market reports and public databases such as the Global Initiative on Sharing All Influenza Database (GISAID) that maintains a record of SARS-CoV-2 mutations to provide quality results and accuracy for our customers. To date, while the exact prevalence of the 69-70del mutation in the SARS-CoV-2 infected population is unknown, preliminary analysis suggests that this variant is significantly more transmissible than previously circulating variants [2] and the prevalence rates are constantly changing.

Since our assay utilizes the S gene as one of the targets for detection of SARS-CoV-2, this FAQ document will address how the S gene mutation might potentially affect our current TaqPath COVID-19 Combo Kit.

## Key facts on the 69-70del S gene mutation and how it may affect our products

- As with nearly all viruses, it is reasonable to expect mutations to naturally occur, and a high rate of mutations in response to selective pressure is especially common for RNA viruses. In light of this, we designed a multi-target assay in order to have built-in redundancy, with three SARS-CoV-2 targets in our TaqPath COVID-19 Combo Kit assay (*orf1-ab*, N gene, and S gene).
- Using the TaqPath COVID-19 Combo Kit as per our Instructions for Use (IFU) and Applied Biosystems™ COVID-19 Interpretive Software program, a positive result is called if at least two of the three SARS-CoV-2 targets are detected. The test results are dictated via our COVID-19 Interpretive Software algorithms that utilize the results from all three targets to generate a final call.
- To date, the 69-70del S gene mutation has not been found to impact test results obtained using the TaqPath COVID-19 Combo Kit. Consequently, no adaptations to the tests or changes in use are necessary at this time. The probability of a mutation in the S gene impacting the accuracy of test results is low (<0.01%).

## FAQs

### 1. When using the TaqPath COVID-19 Combo Kit, what happens if the S gene target “drops out” and only one other target is called positive?

Due to the in-built redundancy of the assay, it is unlikely that the S-gene “drop out” occurs as well as only one of the other two targets (N-gene and *orf1-ab*) are detected. In an unlikely event that only one other target is positive, as per the IFU, the result will be called inconclusive. In case of an inconclusive result, the test needs to be repeated by re-extracting the original sample and repeating the RT-PCR. If the repeat result remains inconclusive, additional testing should be conducted if clinically indicated. Since at least one target was detected, the test will not report out a false negative result.

### 2. What should I do if my results using the TaqPath COVID-19 Combo Kit are inconclusive?

Please refer to the IFU, which provide the following directions:

1. Repeat the test by re-extracting the original sample and repeating the RT-PCR.
2. After retesting one time, report results to the health care provider and appropriate public health authorities.

**Important:** Samples with an inconclusive result for SARS-CoV-2 shall be retested one time. If the repeat result remains inconclusive, the health care provider should conduct additional confirmation testing with a new specimen, if clinically indicated.

If you still need to discuss your specific situation, please contact our technical support at [thermofisher.com/contactus](https://www.thermofisher.com/contactus).

### 3. What are the recommendations for those who are currently using the TaqPath COVID-19 Combo Kit?

Since three SARS-CoV-2 targets are used in our TaqPath COVID-19 Combo Kit and a positive result is called when only two of the three targets are detected

(per our IFU and software algorithm), no adaptations to the test or changes in use are necessary at this time. To date, the 69-70 del S gene mutation has not been found to impact test results obtained using the TaqPath COVID-19 Combo Kit and the probability of a mutation in the S gene impacting the accuracy of test results is low.

### 4. What is the potential impact of the 69-70del mutation on SARS-CoV-2 diagnostics?

The European CDC does not recommend relying only on the S gene for primary detection of SARS-CoV-2 infection [3]. Instead, the European CDC recommends that multi-target RT-PCR assays that include an S gene target affected by the deletions (S gene “drop out”) can be used as a signal for the presence of the 69-70del mutation for further investigation, especially if sequencing capacity is limited. If available, the European CDC recommends confirmation of the 69-70del mutation using sequencing [2].

### 5. What impact might the 69-70del have for labs that use the TaqPath COVID-19 Combo Kit?

The 69-70del in the spike protein results in an S gene “drop out” in RT-PCR assays that use the S gene as one of the targets. An S gene “drop out” does not mean a result is negative; it only indicates that the S gene could not be detected by the assay. Due to the multi-target (N gene, S gene and *orf1-ab*) approach of the TaqPath COVID-19 Combo Kit, the risk of a false negative is low. So far, there are no documented cases of false negative COVID-19 results. While the TaqPath COVID-19 Combo Kit is not intended for use for the detection of the 69-70del mutation in SARS-CoV-2 variant strains, the European CDC recommends that an S gene “drop out” can be used as a signal for the presence of the 69-70del mutant for further investigation, especially if sequencing capacity is limited [2]. Multi-target RT-PCR tests that utilize S gene regions impacted by the 69-70del are quicker and cheaper than sequencing the entire virus and can help keep track of these mutant strains [3].

6. **Do other suppliers' assays target the S gene?**

There are some other suppliers' assays that also target the S gene. However, we are not able to determine the impact of the 69-70del mutation on these assays. While most other assays only target either one or two genes, the TaqPath COVID-19 Combo Kit assay targets three different genes (N gene, S gene, *orf1-ab* gene).

7. **Why was the TaqPath COVID-19 Combo Kit assay designed with the S gene as a target?**

The S-gene was chosen because it was considered a conserved sequence and had the least sequence overlap with other coronaviruses.

8. **What is Thermo Fisher Scientific doing to monitor S-gene mutations in their assays, and will there be product modifications in the future?**

We regularly monitor post-market reports and public database updates to provide quality and accurate products for our customers. To date, there have been no reports of test results being impacted due to the S-gene 69-70del mutation. Consequently, no adaptations to the test or changes in use are necessary at this time.

9. **Who should I reach out to if I have additional questions?**

If you have additional questions or would like to discuss your specific situation, please contact our technical support team. You can identify your local technical support phone number or send an email to technical support at [thermofisher.com/contactus](https://www.thermofisher.com/contactus).

## References

1. COG-UK update on SARS-CoV-2 Spike mutations of special interest. Report 1. Prepared by COG-UK, 20th December 2020. [https://www.cogconsortium.uk/wp-content/uploads/2020/12/Report-1\\_COG-UK\\_19-December-2020\\_SARS-CoV-2-Mutations.pdf](https://www.cogconsortium.uk/wp-content/uploads/2020/12/Report-1_COG-UK_19-December-2020_SARS-CoV-2-Mutations.pdf)
2. Threat Assessment Brief: Rapid increase of a SARS-CoV-2 variant with multiple spike protein mutations observed in the United Kingdom, European Center for Disease Prevention and Control, 20 December 2020. <https://www.ecdc.europa.eu/en/publications-data/threat-assessment-brief-rapid-increase-sars-cov-2-variant-united-kingdom>
3. Mutant coronavirus in the United Kingdom sets off alarms but its importance remains unclear. Kupferschmidt K. doi:10.1126/science.abg2626 <https://www.sciencemag.org/news/2020/12/mutant-coronavirus-united-kingdom-sets-alarms-its-importance-remains-unclear>

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