

# TaqMan SARS-CoV-2 Mutation Panel

## General questions on assays

**Q:** How many Applied Biosystems™ TaqMan™ SARS-CoV-2 Mutation Panel assays are available?

**A:** Over 50. Thermo Fisher Scientific is committed to following SARS-CoV-2 variants, and the number of assays available is subject to change.

**Q:** What is new about the TaqMan SARS-CoV-2 Mutation Panel assays?

**A:** We now have additional targets, including those for the Omicron variant (B.1.1.529) and sub-lineages including BA.1, BA.2, BA.3, BA.4, and BA.5.

**Q:** What is the difference between the premium and additional assays?

**A:** Premium assays are available in regional distribution centers and have shorter lead times. This can range from 1 to 3 days depending on your country. Additional assays are made to order and have longer lead times. Depending on your region, this can range from 5 to 10 days.

**Q:** Are there any changes in the product composition?

**A:** No, the product composition is the same.

**Q:** Should I expect any changes in product performance?

**A:** No, you should not expect any changes in product performance.

**Q:** How does the assay name change impact me?

**A:** We have renamed the assays to reflect more information about the mutations in the SARS-CoV-2 virus. Specifically, the name now includes the full codon change to give more accurate information about the mutations detected. In addition, to be in line with the standard nomenclature for the ORF1 gene, the naming convention has been updated with no changes to the associated AIF files.

## Assay name example—before:

Previously only the nucleotide change was included, as shown below, where C is the wild type nucleotide at the position of the SNP and A is the nucleotide found at the same position in the variant.

orf1.A1708D\_C\_A

## Assay name example—new and improved:

The new assay name includes 3 characters to show the 3 nucleotides found in the reference/wild type codon and the mutant/variant codon, where GCT is the wild type codon at the position of the SNP and GAT is the codon found at the same position in the variant.

ORF1a.A1708D.GCT.GAT

**Q:** How do I find out if a TaqMan SARS-CoV-2 Mutation Panel assay is available in my country?

**A:** Please contact your Thermo Fisher representative to confirm which assays are available in your region.

**Q:** What should I do if the assay I am interested in is not included in the list of TaqMan SARS-CoV-2 Mutation Panel assays?

**A:** Please contact your Thermo Fisher representative to inquire about mutations not included in the current list of assays.

**Q:** How many assays should I run in a 96-well plate? In a 384-well plate?

**A:** In both plate formats, it is important to have at least 6 samples per assay per plate to ensure accurate genotyping analysis. Configuration of plates may vary.

**Q:** Are there any changes to the TaqMan SARS-CoV-2 Mutation Panel workflow?

**A:** There are no changes to the workflow.

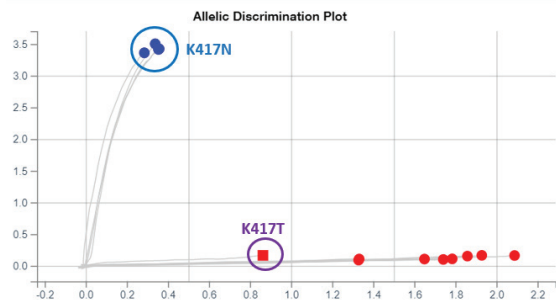
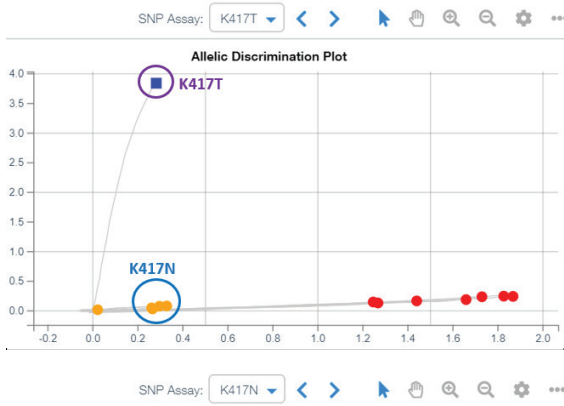
**Q:** Can I combine TaqMan SARS-CoV-2 Mutation Panel assays with other assays that detect changes at the same amino acid position (e.g., K417T/K417N; E484K/E484Q; P681H/P681R)?

**A:** We do not recommend combining assays in the same well.

**Q:** How do I analyze results for the assays detecting mutations leading to changes at the same amino acid position (e.g., K417T/K417N; E484K/E484Q; P681H/P681R)?

**A:** Please refer to the user guide for instructions on how to analyze results for assays detecting mutations at the same amino acid position.

For example, below is an excerpt from the user guide on how to use the K417T and K417N assays.



Samples that are run with the K417T assay and that contain the K417N mutation, or samples run with the K417N assay and that contain the K417T mutation, can cluster near the NTCs or exhibit weak amplification due to weaker binding between the mutation and the wild type probe. If a mutation-containing sample is called as wild type with the adjacent SNP assay, the call can be manually adjusted to “no amp”.

We highly recommend that both K417T and K417N mutation assays be run on the same samples, and that the results of both assays be compared to facilitate accurate genotype analysis.

If it is not possible to make a clear genotype call, rerun the experiment or further characterize the sample by sequence analysis.

**Q:** What real-time PCR instruments are compatible with the TaqMan SARS-CoV-2 Mutation Panel?

**A:** Any Applied Biosystems™ real-time PCR instrument that is compatible with Applied Biosystems™ VIC™ and FAM™ dyes. The assays have been tested internally on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System.

**Q:** What software is recommended for data analysis of the TaqMan SARS-CoV-2 Mutation Panel?

**A:** Applied Biosystems™ QuantStudio™ Design and Analysis Software v2.5 or later.

**Q:** What is the AIF reference file?

**A:** For convenience, Applied Biosystems™ software can import target information directly from assay information files (AIFs, with file extension .aif), which are supplied with assays manufactured by Thermo Fisher.

**Q:** When should I use the AIFs?

**A:** Use the AIFs if you want to annotate the nucleotides associated with the mutant and wild type variant alleles.

**Q:** Where can I obtain the AIFs?

**A:** AIFs can be downloaded by entering the sales order number and rack/plate ID after following instructions at this link: [thermofisher.com/order/taqman-files](https://thermofisher.com/order/taqman-files)

**Q:** Is there a new user guide version available?

**A:** Yes. The most up-to-date version of the user guide can be found at [thermofisher.com/mutationpanel](https://thermofisher.com/mutationpanel) in the “Resources” section.

## General questions on controls

**Q:** Why should I use controls?

**A:** Reference and mutant controls increase the accuracy of the clustering analysis by acting as synthetic templates for the mutation and wild type alleles.

**Q:** Which controls should I include in my experiment?

**A:** Controls may include a no-template control (NTC), reference/wild type control, and positive control.

Possible sources of positive controls that contain the SARS-CoV-2 assay target mutation include the following:

- A sample in your lab that has been characterized by sequence analysis
- A commercial RNA control
- A commercial DNA control (e.g., Invitrogen™ GeneArt™ Strings™ DNA Fragments or plasmids)

Supply the mutation of interest and 100 nucleotides of sequence flanking each side of the mutation. Use approximately 1,000 copies of a control per 20 µL of reaction. Adjust the amount as needed.

**Q:** How many controls should I include in my experiment?

**A:** The recommendation is to use one positive mutant control and one reference control per assay per plate. Configurations may vary. One NTC may also be run per assay per plate so that contamination can be recognized if it occurs.

**Q:** How do I analyze results for controls?

**A:** Set up your analysis to include the mutant control well as a FAM dye–conjugated allele, the reference control well as a VIC dye–conjugated allele, and the NTC well as negative. Proceed with genotyping analysis as recommended in the user guide.

**Q:** How do I order custom Invitrogen™ GeneArt™ controls?

**A:** Please work with your Thermo Fisher representative to select and place an order for custom GeneArt controls for your assays.

**Q:** What is the difference between the GeneArt Strings DNA Fragments and GeneArt DNA plasmids?

**A:** GeneArt DNA plasmids contain primarily mutant amplicons for variants of concern. They are linearized double-stranded DNA in solution at a concentration of  $10^4$  copies/µL. It is recommended that plasmid controls be diluted to  $10^3$  copies/µL for use in the protocol.

GeneArt Strings DNA Fragments are synthesized as double-stranded fragments that can be ordered in lyophilized form. A tube contains  $10^{12}$  copies. GeneArt Strings fragments should be resuspended and diluted to  $10^4$  copies/µL for use in the protocol.

**Q:** How should I prepare the GeneArt Strings fragments?

**A:** Download the product documentation sheet to find the quantity in nanograms. Spin down the dried sample as instructed, and add 1 µL of 1X TE buffer per nanogram of GeneArt Strings DNA, for a solution of  $\sim 10^9$  copies/µL. This can then be diluted to  $10^6$  copies/µL, which can be used as a 100X working stock for addition of 10,000 copies/well. To avoid contaminating assays, aliquot the assay and master mix before pipetting GeneArt Strings DNA.

**Note:** It is important to use best practices to avoid contaminating your laboratory and reagents with GeneArt Strings DNA.

**Q:** What is the number of reactions per tube?

**A:** The minimum yield is 200 ng. The number of reactions varies depending on the input used per well. At 10,000 copies/well there is enough for over 1,000 plates.

**Q:** How long does it take to receive GeneArt DNA plasmid or GeneArt Strings DNA controls?

**A:** GeneArt products should be delivered within 7–14 days, depending on the region.

**Q:** Are there any recommendations for controls from other suppliers?

**A:** Please contact your Thermo Fisher representative for recommendations on controls from other suppliers.

## General questions on the Omicron variant

Q. What is the impact of the Omicron lineages BA.1, BA.1.1, and BA.2 on mutation panels?

A. Tables 1 and 2 summarize the performance impact on our premier and custom assays, respectively.

**Table 1. Premier assays—impact of Omicron lineages BA.1, BA.1.1, and BA.2.**

Mutation	Cat. No.	Effect of mutation in BA.1 and BA.1.1	Expected result	Effect of mutation in BA.2	Expected result
S.D215G.GAT.GGT	A51823	N211I, del212/212	No amplification		
S.D614G.GAT.GGT	A51818				
S.delH69V70	A51808				
S.delY144	A51817	VYY143del	No amplification		
S.E484K.GAA.AAA	A51813	E484A	No amplification	E484A	No amplification
S.E484Q.GAA.CAA	A51820	E484A	No amplification	E484A	No amplification
S.F888L.TTT.CTT	A51827				
S.K417N.AAG.AAT	A51814				
S.K417T.AAG.ACG	A51815	K417N	No amplification	R408S, K417N	No amplification or low VIC*
S.L18F.CTT.TTT	A51830			T19I, LPPA24S	No amplification
S.L452R.CTG.CGG	A51819				
S.N439K.AAC.AAA	A51825				
S.N501Y.AAT.TAT	A51812				
S.P681H.CCT.CAT	A51816	N679K	Lower FAM	N679K	Lower FAM
S.P681R.CCT.CGT	A51822	N679K, P681H	No amplification	N679K, P681H	No amplification
S.S13I.AGT.ATT	A51828			LPPA24S	No amplification or low VIC*
S.S477N.AGC.AAC	A51824	T478K, E484A	No amplification	T478K, E484A	No amplification
S.T20N.ACC.AAC	A51821			T19I, LPPA24S	No amplification
S.V1176F.GTT.TTT	A51826				

\* Low VIC means “possibly unacceptably low”

**Table 2. Custom assays—impact of Omicron lineages BA.1, BA.1.1, and BA.2.**

Mutation	Assay ID	Effect of mutation in BA.1 and BA.1.1	Expected result	Effect of mutation in BA.2	Expected result
Orf1ab.A13057T	CVXGPV9				
orf1.A1708D.GCT.GAT	CVYMJF3	T5386G	No amplification		
S.A222V.GCT.GTT	CVMFVWP				
orf1ab.A2710T	CVZTDZ7				
CoV1.A28272T	CV2W7KZ	A28271T	No amplification	A28271T	No amplification
S.A570D.GCT.GAT	CVU62RC				
S.A701V.GCA.GTA	CVZTDZZ				
N.D3L.GAT.CTA	CVDJXE9				
S.D80A.GAT.GCT	CV32Z6V				
S.delL242.244L	CV2W7KX				
S.E583D.GAG.GAT	CVU62RD				
S.E583D.GAG.GAC	CVWCWCA				
S.EFR156-158G	CVPRJZM	VYY143del	No amplification		
S.F157L.TTC.TTA	CVGZE4Y				
S.F157S.TTC_TCC	CVTZ76G				
S.G339D.GGT_GAT	CV47VRX				
S.G446V.GGT.GTT	CV2W7KY	G446S	No amplification		
S.G769V.GGA.GTA	CVPRJZK				

Table 2. Custom assays—impact of Omicron lineages BA.1, BA.1.1, and BA.2. (continued)

Mutation	Assay ID	Effect of mutation in BA.1 and BA.1.1	Expected result	Effect of mutation in BA.2	Expected result
S.H66D.CAT.GAT	CVCE3VC	A67V, HV69del	No amplification		
ORF1.K1795Q.AAA.CAA	CVAAAAE				
S.L452Q.CTG.CAG	CVKA3AV				
S.N450K.AAT.AAG	CVXGPV7				
S.N460K.AAT.AAA	CV7DPCR				
S.N460K.AAT.AAG	CV9HHWN				
S.N460T.AAT.ACT	CVZTDZ2				
P5743S.CCA_TCA	CVYMJF6				
orf8.Q27ST.CAA.TAA	CVRWEKG				
S.Q493R.CAA_CGA	CVH49P2				
S.Q52R.CAG.CGG	CV47VRU				
S.Q613H.CAG.CAT	CV32Z6W				
S.Q677H.CAG.CAT	CVRWEKH	N679K	Lower VIC**	N679K	Lower VIC**
S.Q677H.CAG.CAC	CVTZ76F	N679K	Lower VIC**	N679K	Lower VIC**
S.R246I.AGA.ATA	CV7DPCP				
S.R346K.AGA_AAA	CVZTDZ3				
S.S982A.TCA.GCA	CVXGPV6	N969K, L981F	No amplification		
S.Y145H.TAC.CAC	CVFVKJ7	VYY143del	No amplification		Lower VIC**
T1055A.ACA_GCA	CV32Z6X				
orf1ab.T13195C	CVWCWCF				
S.T19R.ACA.AGA	CVXGPWD			T19I	No amplification
S.T478K.ACA.AAA	CVNKRFP	S477N, T478K, E484A	No amplification	S477N, T478K, E484A	No amplification
S.T547K.ACA.AAA	CVYMJGA				
S.T716I.ACA.ATA	CVWCWA9				
S.T95I.ACT_ATT	CVRWEKJ				
S.V367F.GTC.TTC	CVEPRY6	S371L, S373P, S375F	No amplification or low VIC*	S371F, S373P, S375F, T376A	No amplification or low VIC*
S.W152L.TGG.TTG	CVH49PW	VYY143del	No amplification		
S.Y453F.TAT.TTT	CVNKRFM	N440K, G446S	No amplification		

\* Low VIC means "possibly unacceptably low".

\*\* Lower VIC means "acceptably low".

Q. Where can I find the updated list of assays?

A. We have updated our assay selection list at [thermofisher.com/mutationpanel](https://www.thermofisher.com/mutationpanel)

Q. What if an assay I used is no longer present on the website?

A. Please contact your sales representative for additional support.

Q. Is there any impact from the Omicron variant and its sublineages on the protocol?

A. The protocol and the recommended reagents remain identical, including the Applied Biosystems™ TaqPath™ 1-Step Multiplex Master Mix.

Find out more at [thermofisher.com/mutationpanel](https://www.thermofisher.com/mutationpanel)