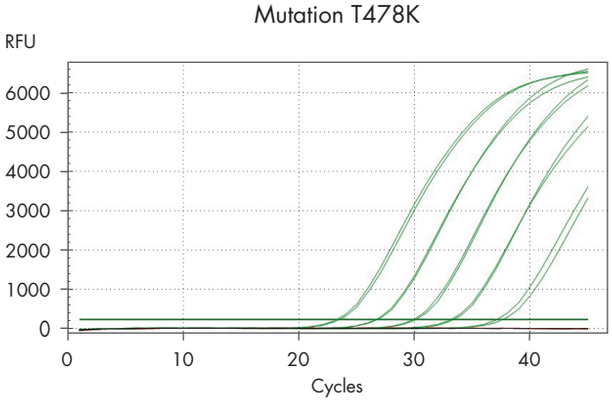
Assay Profile

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&amp® 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**), 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.

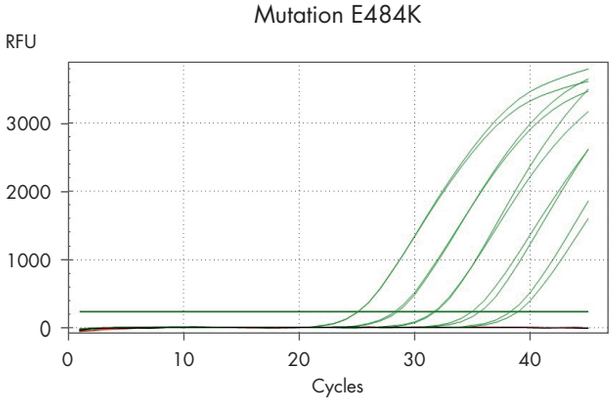
Mutation T478K



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

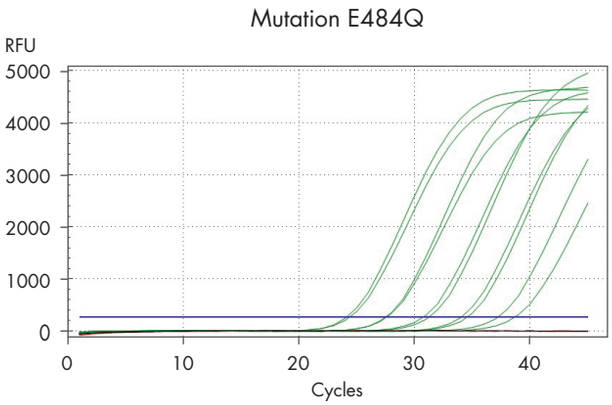
Specific and sensitive detection of the mutation T478K. In green: amplification curves from dilution series (10^5–10^1 cp/rxn, in duplicates) of an in-vitro transcript (IVT) bearing the SARS-CoV-2 mutation T478K. In red: amplification curve of an IVT of the corresponding WT sequence from SARS-CoV-2 at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

Mutation E484K



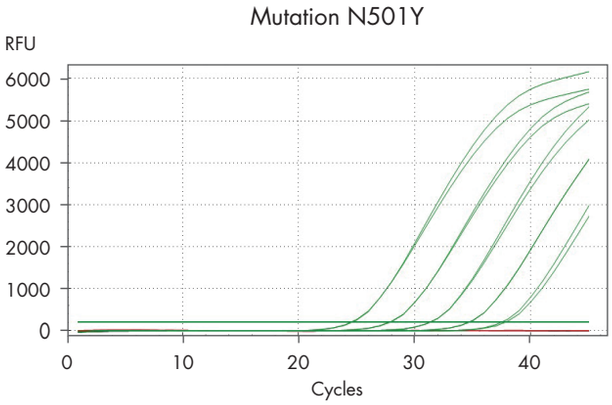
IVT copies/ reaction	Bio-Rad CFX	ABI QuantStudio	QIAGEN Rotor-Gene Q
	Ct		
10^5	25.1	25.6	25.1
10^4	28.3	29.3	28.5
10^3	31.6	33.4	32.0
10^2	35.2	36.7	35.6
10^1	38.5	41.3	39.1

Specific and sensitive detection of the mutation E484K. 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 E484K. 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.



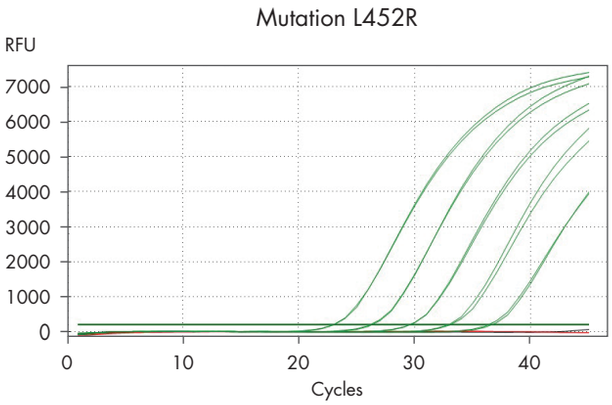
IVT copies/ reaction	Bio-Rad CFX	ABI QuantStudio	QIAGEN Rotor-Gene Q
		Ct	
10^5	24.3	24.4	24.2
10^4	27.6	27.8	27.6
10^3	31.0	31.1	30.9
10^2	34.3	34.8	34.1
10^1	38.0	39.3	38.1

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.



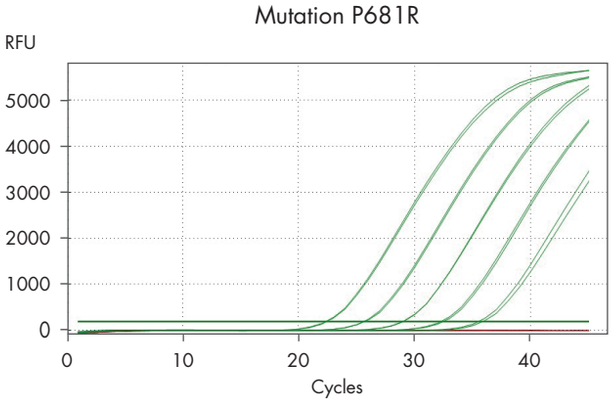
IVT copies/ reaction	Bio-Rad CFX	ABI QuantStudio	QIAGEN Rotor-Gene Q
		Ct	
10^5	24.5	24.4	24.12
10^4	27.8	27.8	27.25
10^3	31.2	31.1	31.01
10^2	34.7	34.4	33.99
10^1	37.6	37.6	38.21

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.



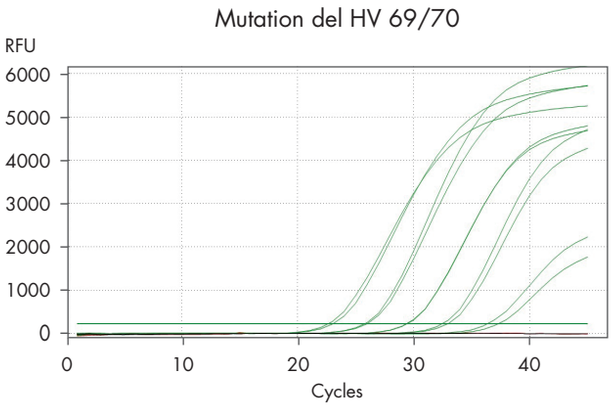
IVT copies/ reaction	Bio-Rad CFX	ABI QuantStudio	QIAGEN Rotor-Gene Q
		Ct	
10^5	22.9	22.6	22.72
10^4	26.2	25.8	25.28
10^3	29.5	29.1	28.98
10^2	33.0	32.4	32.65
10^1	36.6	35.7	35.83

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.



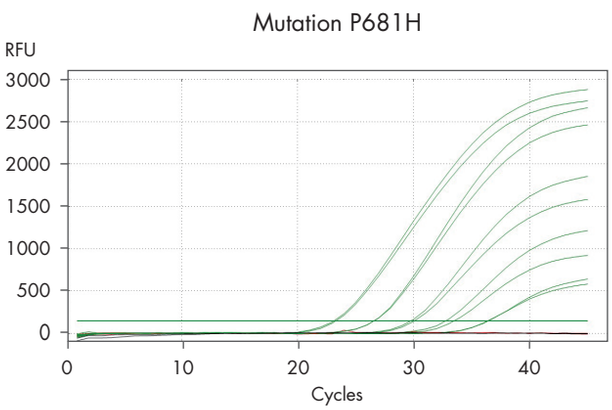
IVT copies/ reaction	Bio-Rad CFX	ABI QuantStudio	QIAGEN Rotor-Gene Q
	Ct		
10^5	22.4	22.30	23.0
10^4	25.7	25.63	25.9
10^3	29.0	29.02	29.3
10^2	32.4	32.31	33.2
10^1	35.4	35.50	36.1

Specific and sensitive detection of the mutation P681R. 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 P681R. 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.



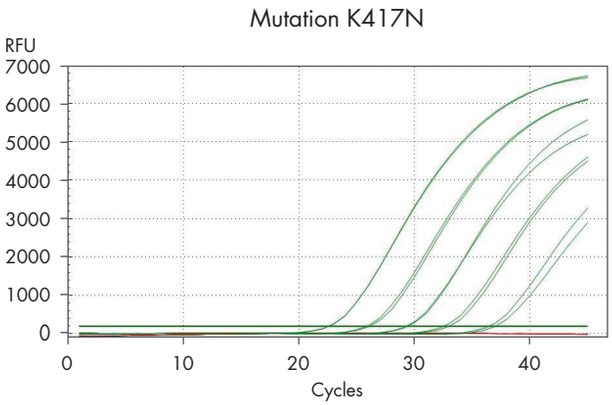
IVT copies/ reaction	Bio-Rad CFX	ABI QuantStudio	QIAGEN Rotor-Gene Q
	Ct		
10^5	21.47	21.67	21.57
10^4	24.80	24.65	24.82
10^3	27.83	28.26	27.80
10^2	31.62	31.52	31.49
10^1	34.24	35.64	35.04

Specific and sensitive detection of the deletion mutation HV 69/70. In green: amplification curves from dilution series (10^5–10^1 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 10^7 cp/rxn. In black (overlapping with the red line) is the NTC.



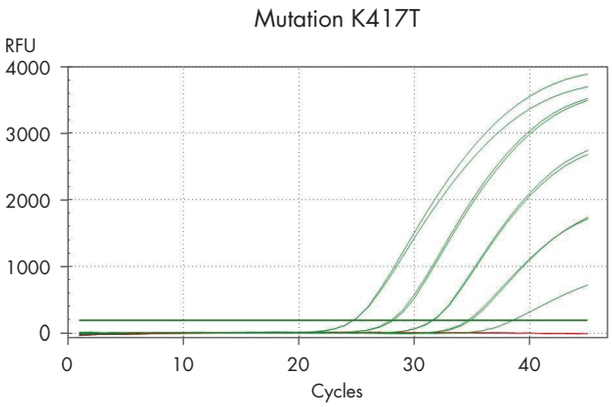
IVT copies/ reaction	Bio-Rad CFX	ABI QuantStudio	QIAGEN Rotor-Gene Q
	Ct		
10^5	23.18	23.17	24.17
10^4	26.37	26.54	27.13
10^3	29.64	29.87	29.53
10^2	32.77	33.08	32.85
10^1	35.70	36.34	37.46

Specific and sensitive detection of the mutation P681H. 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 P681H. 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.



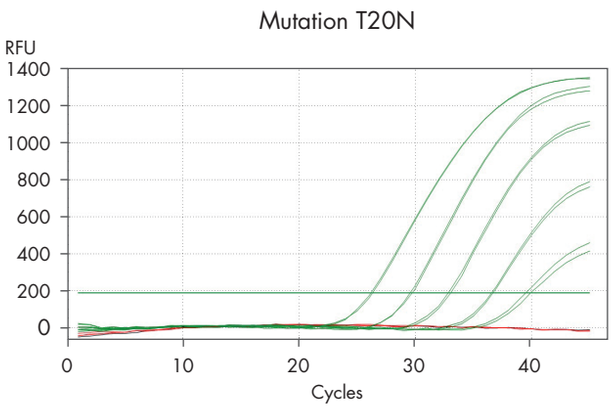
IVT copies/ reaction	Bio-Rad CFX	ABI QuantStudio	QIAGEN Rotor-Gene Q
	Ct		
10^5	22.4	22.4	22.3
10^4	26.0	25.8	25.9
10^3	29.3	29.2	29.3
10^2	32.7	32.5	32.7
10^1	36.2	37.0	36.6

Specific and sensitive detection of the mutation K417N. 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 K417N. 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/ reaction	Bio-Rad CFX	ABI QuantStudio	QIAGEN Rotor-Gene Q
	Ct		
10^5	24.5	24.2	24.3
10^4	27.9	27.7	27.5
10^3	31.4	31.1	31.1
10^2	34.6	34.8	34.8
10^1	39.7	39.3	39.4

Specific and sensitive detection of the mutation K417T. 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 K417T. 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/ reaction	Bio-Rad CFX	ABI QuantStudio	QIAGEN Rotor-Gene Q
	Ct		
10^5	26.3	26.4	26.49
10^4	29.6	29.9	30.19
10^3	33.0	33.6	33.20
10^2	36.5	37.3	37.97
10^1	39.7	42.1	45.00

Specific and sensitive detection of the mutation T20N. 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 T20N. 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.

Genotyping assay compatibility with control genes in a duplex reaction

	Control genes			
Mutation assays	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 at **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.**

Download a Quick-Start Protocol for the SARS-CoV-2 LNA qPCR Assays at **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.