



# PrimePCR SARS-CoV-2 Multiple and Single Mutation Assays



- SARS-CoV-2 surveillance by RT-qPCR — profile the mutation fingerprint of positive samples
- Rapid time to results — <60 minute protocol for use by researchers
- Flexible assay formats — multiple and single mutation detection
- High sensitivity — RT-qPCR assays can detect mutant alleles in samples at low RNA levels
- Expanding menu — assay options can be updated as new mutations and variants arise

Identify currently relevant mutations in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to rapidly screen samples for multiple variants of interest or concern, or survey the prevalence of a specific mutation using reverse transcription quantitative PCR (RT-qPCR) in a research use only (RUO) format.

The PrimePCR SARS-CoV-2 Multiple Mutation Assays contain a primer set, up to six differentially labeled probes with homology to specific spike protein gene mutations, and an internal RNA integrity control. When used in combination with SARS-CoV-2 Standard and Variant Controls from Exact Diagnostics (a Bio-Rad Laboratories, Inc. company), the assays can be used by researchers to rapidly profile the molecular fingerprint of RNA from prior sample extractions. The SARS-CoV-2 Variant and SARS-CoV-2 Standard (wild-type) positive controls can be included to monitor reverse transcription, amplification, and detection steps. The high sensitivity of these assays can

be validated by researchers for use in assessing low-level samples with quantification cycle (Cq) values up to 40.

PrimePCR SARS-CoV-2 Single Mutation Assays target specific individual mutations in the SARS-CoV-2 genome that are believed to confer an evolutionary advantage to the virus. Assays contain a primer set plus two probes: one probe (labeled with HEX) matches the reference genome and the other probe (labeled with FAM) matches the mutation. The presence or absence of a specific mutation can be assessed in extracted SARS-CoV-2-positive samples using allelic discrimination. Each assay also contains a primer set and a probe as an internal control to assess the RNA integrity of the sample.

As part of an ongoing surveillance program, Bio-Rad can add new assays as novel mutations are acquired by the virus. Visit [bio-rad.com/SC2Variants](https://www.bio-rad.com/SC2Variants) to view the complete list of assays available.

Feature	PrimePCR SARS-CoV-2 Multiple Mutation Assay	PrimePCR SARS-CoV-2 Single Mutation Assay
Assay format (RUO)	Primer set with up to 6 probes	Primer set with reference and mutant allele probes
Mutations detected	Up to 5	1
Fluorophores	FAM HEX Texas Red ATTO 647 Cy5.5	FAM HEX Texas Red
Internal RNA integrity control	Probe to a conserved region in spike gene between the primers (multiplexed control [MPC])	Primer set and a probe to <i>RPP40</i>
Protocol time	<60 minutes	
Product sizes available	200 reactions 1,000 reactions 2,500 reactions	

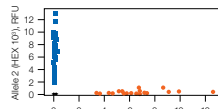
### Assay Workflows

Prepare reactions with remnant-extracted RNA plus controls

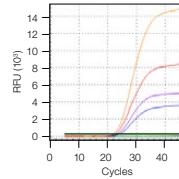


Analyze

PrimePCR SARS-CoV-2 Single Mutation Assays

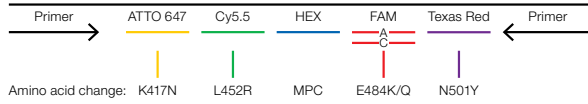


PrimePCR SARS-CoV-2 Multiple Mutation Assays



Interpret

#### Assay 1

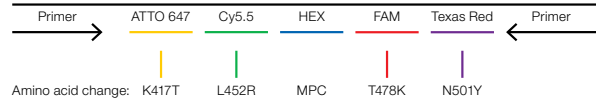


Amino acid change: K417N L452R MPC E484K/Q N501Y

SARS-CoV-2 Variant	K417N	L452R	MPC	E484K	E484Q	N501Y
Alpha (B.1.1.7)			•			•
Beta (B.1.351)	•		•	•		•
Delta (B.1.617.2)		•	•			
Epsilon (B.1.427/B.1.429)		•	•			
Gamma (P.1)			•	•		
Kappa (B.1.617.1/B.1.617.3)		•	•		•	
Lambda (C.37)			•			
Omicron (B.1.1.529)	•		•			•*

\* Omicron has the N501Y mutation but it may not be detected due to other mutations in the region.

#### Assay 2

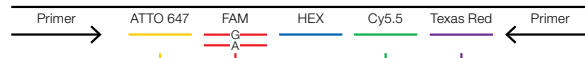


Amino acid change: K417T L452R MPC T478K N501Y

SARS-CoV-2 Variant	K417T	L452R	MPC	T478K	N501Y
Alpha (B.1.1.7)			•		•
Beta (B.1.351)			•		•
Delta (B.1.617.2)		•	•	•	
Epsilon (B.1.427/B.1.429)		•	•		
Gamma (P.1)	•		•		
Kappa (B.1.617.1/B.1.617.3)		•	•		
Lambda (C.37)			•		
Omicron (B.1.1.529)			•	•*	•*

\* Omicron has the T478K and N501Y mutations but they may not be detected due to other mutations in the region.

#### Assay 3



Amino acid change: K417N L452R/Q MPC T478K F490S

SARS-CoV-2 Variant	K417N	L452R	L452Q	MPC	T478K	F490S
Alpha (B.1.1.7)				•		
Beta (B.1.351)	•			•		
Delta (B.1.617.2)		•		•	•	
Epsilon (B.1.427/B.1.429)		•		•		
Gamma (P.1)				•		
Kappa (B.1.617.1/B.1.617.3)		•		•		
Lambda (C.37)			•	•		•
Omicron (B.1.1.529)	•			•	•*	

\* Omicron has the T478K mutation but it may not be detected due to other mutations in the region.

## PrimePCR SARS-CoV-2 Multiple Mutation Assay Design

Mutation profiling can be used to assess the probability that a sample contains a SARS-CoV-2 variant of interest or concern. PrimePCR SARS-CoV-2 Multiple Mutation Assays include probes with homology to the sequences that encode the following key spike protein mutations.

### Assay 1

SARS-CoV-2 Variant	K417N	L452R	MPC	E484K	E484Q	N501Y
Alpha (B.1.1.7)			•			•
Beta (B.1.351)	•		•	•		•
Delta (B.1.617.2)		•	•			
Epsilon (B.1.427/B.1.429)		•	•			
Gamma (P.1)			•	•		
Kappa (B.1.617.1/B.1.617.3)		•	•		•	
Lambda (C.37)			•			
Omicron (B.1.1.529)	•		•			•*

MPC, multiplexed control.

\* Omicron has the N501Y mutation but it may not be detected due to other mutations in the region.

### Assay 2

SARS-CoV-2 Variant	K417T	L452R	MPC	T478K	N501Y
Alpha (B.1.1.7)			•		•
Beta (B.1.351)			•		•
Delta (B.1.617.2)		•	•	•	
Epsilon (B.1.427/B.1.429)		•	•		
Gamma (P.1)	•		•		
Kappa (B.1.617.1/B.1.617.3)		•	•		
Lambda (C.37)			•		
Omicron (B.1.1.529)			•	•*	•*

MPC, multiplexed control.

\* Omicron has the T478K and N501Y mutations but they may not be detected due to other mutations in the region.

### Assay 3

SARS-CoV-2 Variant	K417N	L452R	L452Q	MPC	T478K	F490S
Alpha (B.1.1.7)				•		
Beta (B.1.351)	•			•		
Delta (B.1.617.2)		•		•	•	
Epsilon (B.1.427/B.1.429)		•		•		
Gamma (P.1)				•		
Kappa (B.1.617.1/B.1.617.3)		•		•		
Lambda (C.37)			•	•		•
Omicron (B.1.1.529)	•			•	•*	

MPC, multiplexed control.

\* Omicron has the T478K mutation but it may not be detected due to other mutations in the region.

## Related Products

Catalog # Description

### Single Mutation Assays

12016270 PrimePCR SARS-CoV-2 Variant Assay, 200 reactions  
 12016287 PrimePCR SARS-CoV-2 Variant Assay 1,000 reactions  
 12016286 PrimePCR SARS-CoV-2 Variant Assay, 2,500 reactions

### Multiple Mutation Assays

12016711 PrimePCR SARS-CoV-2 Variant Multi Kit, 200 reactions  
 12016731 PrimePCR SARS-CoV-2 Variant Multi Kit, 1,000 reactions  
 12016689 PrimePCR SARS-CoV-2 Variant Multi Kit, 2,500 reactions

### Exact Diagnostics SARS-CoV-2 Reference Control (RUO)

COV019 SARS-CoV-2 Standard

### Exact Diagnostics SARS-CoV-2 Variant Mutation Controls (RUO)

COVA SARS-CoV-2 S Gene Alpha Variant Control (B.1.1.7)  
 COVB SARS-CoV-2 S Gene Beta Variant Control (B.1.351)  
 COVE SARS-CoV-2 S Gene Epsilon Variant Control (B.1.427 and B.1.429)  
 COVG SARS-CoV-2 S Gene Gamma Variant Control (P.1)  
 COVDK SARS-CoV-2 S Gene Delta-Kappa Variants Control (Delta B.1.617.2 and AY sublineages, Kappa B.1.617.1 and B.1.617.3)

### Exact Diagnostics SARS-CoV-2 Negative Control (RUO)

COV000 SARS-CoV-2 Negative

### Instruments

12011319 CFX Opus 96 Real-Time PCR System  
 12011452 CFX Opus 384 Real-Time PCR System  
 1855195 CFX96 Touch Real-Time PCR System  
 1855485 CFX384 Touch Real-Time PCR System  
 1845097-IVD, 1841000-IVD CFX96 Dx Real-Time PCR System

### Reagents and PCR Plastics

12010176 Reliance One-Step Multiplex RT-qPCR Supermix, 1 ml, 200 x 20 µl reactions  
 HSP9955 or equivalent\* Hard-Shell 96-Well PCR Plates, low profile, thin wall, skirted, white shell/white well, barcoded, pkg of 50  
 HSP3805 or equivalent\* Hard-Shell 384-Well PCR Plates, low profile, thin wall, skirted, clear shell/white well, pkg of 50  
 MSB1001 Microseal 'B' PCR Plate Sealing Film, adhesive, optical, pkg of 100

\* Refer to the Hard-Shell PCR Plates Brochure (bulletin 5496) for other 96- and 384-well colored shell/white well PCR plates.



Visit [bio-rad.com/SC2Variants](https://bio-rad.com/SC2Variants) for more information.



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