

## Detection Kit for 6 mutations in S gene of SARS-CoV-2(ARMS-PCR)

### Amendment to Instructions for Use

#### 【Product name】

Detection Kit for 6 mutations in S gene of SARS-CoV-2(ARMS-PCR)

#### 【Product specifications】

50 tests/kit

#### 【Catalogue number】

PGI030019

#### 【Intended use】

The kit is a qualitative *in vitro* nucleic acid amplification assay to detection the mutations of N501Y, A570D, HV69-70del, K417N, K417T, and E484K in S gene of SARS-CoV-2 in throat swab or sputum specimen confirmed SARS-CoV-2 positive by RT-PCR.

The amendment was to interpret the testing results for determining and differentiating SARS-CoV-2 variants.

#### 【Quality control】

- Blank Control:

PCR-Mix1: Ct values at FAM, VIC/HEX and ROX channels are 0 or no data available. Ct value at CY5 channel is 0, no data available or higher than 38.

PCR-Mix2: Ct values at FAM, CY5, ROX and VIC/HEX channels are 0 or no data available.

- Positive Control:

PCR-Mix1 and 2: Ct values at FAM, CY5, ROX and VIC/HEX channels are all in S-shape with Ct values no higher than 35 in both PCR-Mix1 and Mix2.

- Above requirements should be met in a single test. Otherwise, the test is invalid. Please operate the retest strictly in line with the package insert.

#### 【Threshold and reference range】

- Reference range of the kit was determined based on the Receiver Operating Characteristic curve and percentile method. Cut-off values for positive N501Y, A570D, HV69-70del, K417N, K417T, and E484K are Ct values lower than 41.
- $\Delta$  Ct calculated using formula: Ct value of mutation- Ct value of ORF1ab. Please refer to table 1 below for each testing mutation.

Table 1  $\Delta$  Ct calculation method

$\Delta$ Ct	Allele	Fluorescent signal	$\Delta$ Ct value
/	ORF1ab.	FAM(PCR-MIX1)	/
$\Delta$ Ct1	N501Y	VIC(PCR-MIX1)	Ct <sub>N501Y</sub> - Ct <sub>ORF1ab</sub>
$\Delta$ Ct2	K417N	ROX(PCR-MIX1)	Ct <sub>K417N</sub> - Ct <sub>ORF1ab</sub>
$\Delta$ Ct3	E484K	FAM (PCR-MIX2)	Ct <sub>E484K</sub> - Ct <sub>ORF1ab</sub>
$\Delta$ Ct4	HV69-70del	VIC/HEX(PCR-MIX2)	Ct <sub>HV69-70del</sub> - Ct <sub>ORF1ab</sub>
$\Delta$ Ct5	K417T	ROX(PCR-MIX2)	Ct <sub>K417T</sub> - Ct <sub>ORF1ab</sub>
$\Delta$ Ct6	A570D	CY5(PCR-MIX2)	Ct <sub>A570D</sub> - Ct <sub>ORF1ab</sub>

- Cut-off value for internal reference was determined as 38, no higher than 38 as positive.
- The identification of mutant strain of N501Y, HV69-70del, E484K, A570D, K417N and K417T should be determined by Ct values in combination with  $\Delta Ct$ .

Table 2 Reference range for different mutation .

Allele	$\Delta Ct$ value	Result
N501Y	$\Delta Ct1 \leq 6$	N501Y (mutant strain)
	$\Delta Ct1 > 6$	Non-N501Y mutant
K417N	$\Delta Ct2 \leq 6$	K417N (mutant strain)
	$\Delta Ct2 > 6$	Non-K417N mutant
E484K	$\Delta Ct3 \leq 6$	E484K (mutant strain)
	$\Delta Ct3 > 6$	Non-E484K mutant
HV69-70del	$\Delta Ct4 \leq 6$	HV69-70del (mutant strain)
	$\Delta Ct4 > 6$	Non- HV69-70del mutant
K417T	$\Delta Ct5 \leq 6$	K417T (mutant strain)
	$\Delta Ct5 > 6$	Non-K417T mutant
A570D	$\Delta Ct6 \leq 6$	A570D (mutant strain)
	$\Delta Ct6 > 6$	Non-A570D mutant

- Cut-off value for internal reference was determined as 38, no higher than 38 as positive.

**【Testing result interpretation】**

Positive mutations or mutation combinations could be used to determine the SARS-CoV-2 variants as table below 3.

Table 3 Summary of testing result interpretation for SARS-CoV-2 variants

Variant	Mutation positive						Result interpretation
	HV69-70 del	K417N	K417T	E484K	N501Y	A570D	
Alpha	Pos				Pos	Pos	Positive Alpha
Beta		Pos		Pos	Pos		Positive Beta
Gamma		Pos	Pos	Pos			Positive Gamma
Delta							
Omicron	Pos	Pos			Pos		Positive Omicron

- The sample was positive Alpha variant of SARS-CoV-2 when HV69-70 del, N501Y and A570D were all tested positive.
- The sample was positive Beta variant of SARS-CoV-2 when K417N, E484K and N501Y were all tested positive.
- The sample was positive Gamma variant of SARS-CoV-2 when K417N, K417T and E484K were all tested positive.
- The sample was positive Omicron variant of SARS-CoV-2 when HV69-70 del, K417N and N501Y were all tested positive.

