INTENDED USE

PANAMutyper™ R EGFR is an *in vitro* diagnostic test kit to detect and genotype somatic mutations in EGFR oncogene (Table 1). The kit is developed to be used by the trained laboratory professionals, within the fully equipped laboratory environment, using the DNA derived from plasma, serum, body fluid and formalin-fixed paraffin-embedded (FFPE) tissue of non-small cell lung cancer (NSCLC) patients.

The PANAMutyper™ R EGFR is a CE marked diagnostic device in accordance with the European Union *in vitro* Diagnostic Medical Device Directive 98/79/EC.
It is MFDS approved for clinical use in Korea.

Table 1. EGFR mutations detected and genotyped by PANAMutyper™ R EGFR

No.	Reagent	Dye	Exon	Amino Acid Change	Nucleotide change	Cosmic No.
1	G719X/ S768I	FAM	18	p.G719A	c.2156G>C	6239
2				p.G719S	c.2155G>A	6252
3				p.G719C	c.2155G>T	6253
4		HEX (or VIC)	20	p.S768I	c.2303G>T	6241
5	E19del/ E20ins A/EIC	HEX (or VIC)	19	p.E746_A750delELREA	c.2235_2249 del 15	6223
6				p.E746_A750delELREA	c.2236_2250 del 15	6225
7				p.E746_T751>IP	c.2235_2251 >AATTC	13552
8				p.K745_E749delKELRE	c.2233_2247 del15	26038
9				p.E746_T751>I	c.2235_2252 >AAT (complex)	13551
10				p.E746_A750>IP	c.2235_2248 >AATTC	13550
11				p.L747_S752>Q	c.2239_2256 >CAA	12403
12				p.S752_I759delSPKANKEI	c.2253_2276 del 24	13556
13				p.E746_T751>VA	c.2237_2253>TTGC T	12416
14				p.E746_T751>A	c.2237_2251 del 15	12678
15				p.L747_T751>Q	c.2238_2252 >GCA(complex)	12419
16				p.L747_T751delLREAT	c.2240_2254 del 15	12369
17				p.L747_T751delLREAT	c.2239_2253 del 15	6254
18				p.E746_T751>V	c.2237_2252 >T	12386
19				p.E746_S752>I	c.2235_2255 >AAT	12385
20				p.L747_T751delLREAT	c.2238_2252 del 15	23571

PANAGENE

PANAMutyper R EGFR

No.	Reagent	Dye	Exon	Amino Acid Change	Nucleotide change	Cosmic No.
21	E19del/ E20ins A/EIC	HEX (or VIC)	19	p.E746_T751delELREAT	c.2236_2253 del 18	12728
22				p.E746_S752>A	c.2237_2254 del 18	12367
23				p.E746_S752>V	c.2237_2255>T (complex)	12384
24				p.E746_S752>D	c.2238_2255 del 18	6220
25				p.L747_A750>P	c.2238_2248 >GC(complex)	12422
26				p.L747_E749delLRE	c.2239_2247 delTTAAGAGAA	6218
27				p.L747_S752delLREATS	c.2239_2256 del 18	6255
28				p.L747_ A750>P	c.2239_2248 TTAAGAGAAG>C	12382
29				p.L747_P753>Q	c.2239_2258 >CA(complex)	12387
30				p.L747_T751>S	c.2240_2251 del 12	6210
31				p.L747_P753>S	c.2240_2257 del 18	12370
32				p.L747_T751>P	c.2239_2251 >C(complex)	12383
33				p.E746_P753>VS	c.2237_2257 >TCT	18427
34		ROX	20	p.D770_N771insG	c.2310_2311 insGGT	12378
35				p.P772_H773insTTP	c.2315_2316 insGACAACCCC	-
36				p.P772_H773insGNP	c.2315_2316 insGGGCAACCC	-
37				p.V769_D770insASV	c.2309_2310AC>CC AGCGTGGAT	13558
38	E20ins B	ROX	20	p.V769_D770insASV	c.2307_2308 insGCCAGCGTG	12376
39				p.H773_V774insH	c.2319_2320 insCAC	12377
40				p.H773L	c.2318 A>T	13005
41				p.H773_V774insPH	c.2319_2320 insCCCCAC	12380
42				p.V774_C775insHV	c.2321_2322 insCCACGT	18432
43				p.D770_N771insSVD	c.2311_2312 insGCGTGGACA	13428
44	T790M	HEX (or VIC)	20	p.T790M	c.2369C>T	6240
45	L858R	ROX	21	p.L858R	c.2573T>G	6224
46				p.L858R	c.2573_2574 TG>GT	12429
47	L861Q	ROX	21	p.L861Q	c.2582T>A	6213

* All listed EGFR mutations can be genotyped except E19del and E20ins.
* Cosmic Numbers are taken from 'The Catalogue of Somatic Mutations in Cancer' (http://cancer.sanger.ac.uk/cosmic)

PRINCIPLE AND OVERVIEW

The PANAMutyper™ R EGFR is based on peptide nucleic acid (PNA)-mediated real-time PCR clamping and melting peak analysis technology. By using wild type DNA specific PNA clamp probe, the amplification of the wild type DNA is suppressed. In addition, by using mutant type DNA specific PNA detection probe which has fluorescent dye and quencher, EGFR mutation can be genotyped by melting peak analysis.

Therefore, it is possible to genotype EGFR mutations accurately with very high detection sensitivity for only a minute amount of mutant type DNA. It is also very fast and convenient method for detecting mutant through the PCR reaction.

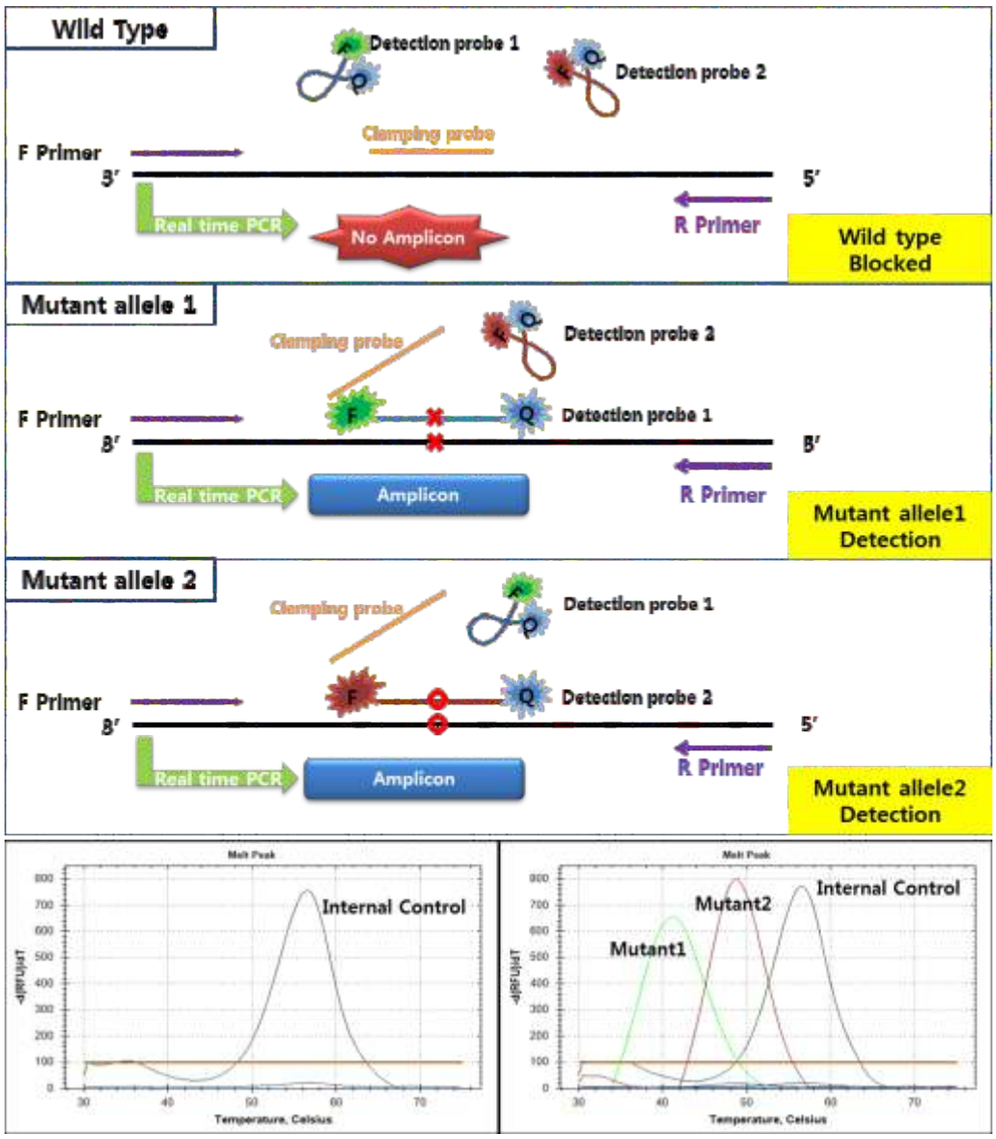


Figure 1. Principle of the PANAMutyper™ R EGFR

EQUIPMENT AND MATERIALS SUPPLIED BY THE USER

- ✓ Reagents and equipment for DNA extraction
- ✓ Pipettes (capacity 10 µl, 20 µl, and 200 µl)
- ✓ Filter pipette tips
- ✓ Bench top microcentrifuge
- ✓ Vortex mixer
- ✓ Disposable gloves, powder-free
- ✓ DNase-free PCR tubes.

◆ **It is recommended to use below PCR system and plastic consumables for the best performance.**

- ➢ White PCR plate (Catalog No. BRMLL-9651, Bio-Rad)
- ➢ 96 well plate (Catalog No. N8010560, ABI)
- ➢ Adhesive seals (Catalog No. MSB-1001, Bio-Rad)
- ➢ Adhesive film (Catalog No. 4311971, ABI)
- ➢ Real-time PCR system

Table 2. Compatible real time PCR instruments

Company	Model
Bio-Rad	CFX 96 real-time PCR detection system
Applied Biosystems	QuantStudio® 5

WARNINGS AND PRECUATIONS

- ✓ **Please read carefully this instruction and become familiar with all components of the kit prior to use.**
- ✓ **PANAMutyper™ R EGFR is for *in vitro* diagnostic use.**
- ✓ This kit should be used by trained laboratory professionals.
- ✓ All experiments should be performed under proper clean conditions in order to prevent contamination. It is recommended that a user has separate, dedicated pipettes and filter pipette tips to add DNA template and prepare PCR reagents.
- ✓ Always wear powder-free gloves when you handle the kit.
- ✓ To avoid repeated freezing and thawing, aliquot all reagents into appropriate volumes and store frozen until use. Thaw appropriate volumes of reagents before each experiment.
- ✓ All experimental procedures should be performed at room temperature. However, please minimize exposure time of Taq DNA polymerase at room temperature for the optimal amplification.
- ✓ Dissolve reagents completely and mix them thoroughly by vortex.
- ✓ Tubes should be briefly centrifuged before use.
- ✓ Tubes containing PNA probe should be protected from prolonged exposure to light.
- ✓ Use only recommended instrument and consumables only (page 7). If not, it may cause loss of performance and increase the chance of false result.
- ✓ Additional validation testing by a user may necessary when non-recommended instrument is used.
- ✓ Do not use incorrect volume of reagent or target DNA; it may cause loss of performance and increase the chance of false result.
- ✓ Do not interchange or mix reagents from different lots or other manufacture's product.
- ✓ Do not re-use any remaining reagents after PCR amplification is complete.
- ✓ Do not use the reagents after their expiration date.
- ✓ If the melting peak of internal control for the DNA (extracted from samples) does not appear, it is possible that the amplification efficiency of the sample is low and it can cause an error in the assessment.
- ✓ If the DNA is extracted from a FFPE sample, it may require further purification depending on the performance of the DNA extraction kit.

STORAGE CONDITION AND STABILITY

The PANAMutyper™ R EGFR is shipped on ice packages and must still be frozen on arrival. If the kit is not frozen on arrival please contacts PANAGENE or the local distributor (see back cover).

The PANAMutyper™ R EGFR should be stored immediately upon receipt at -15℃ to -20℃. When stored under the recommended storage conditions, the kit is stable until the labeled expiration date.

After open the kit, reagents can be stored in their original packaging at -15℃ to -20℃ for 90 days or until the expiration date, whichever comes first.

KIT CONTENTS

A total of 24 samples can be tested using a kit.

Table 3. Reagents provided in the PANAMutyper™ R EGFR

No.	Name of component	Description	Volume	Cap label
1	G719X/S768I	G719X/S768I PNA probe and primers	550 µl	M.EGFR 1
2	E19del/E20ins A/EIC	E19del/E20ins A/EIC PNA probe and primers	550 µl	M.EGFR 2
3	E20ins B	E20ins B PNA probe and primers	550 µl	M.EGFR 3
4	T790M	T790M PNA probe and primers	550 µl	M.EGFR 4
5	L858R	L858R PNA probe and primers	550 µl	M.EGFR 5
6	L861Q	L861Q PNA probe and primers	550 µl	M.EGFR 6
7	Taq DNA polymerase	Taq DNA polymerase	90 µl/vial, 2 vials	Taq
8	Negative control	Negative control	400 µl	NC
9	Positive control	Positive control	400 µl	PC

* EIC: EGFR Internal Control

PROCEDURES

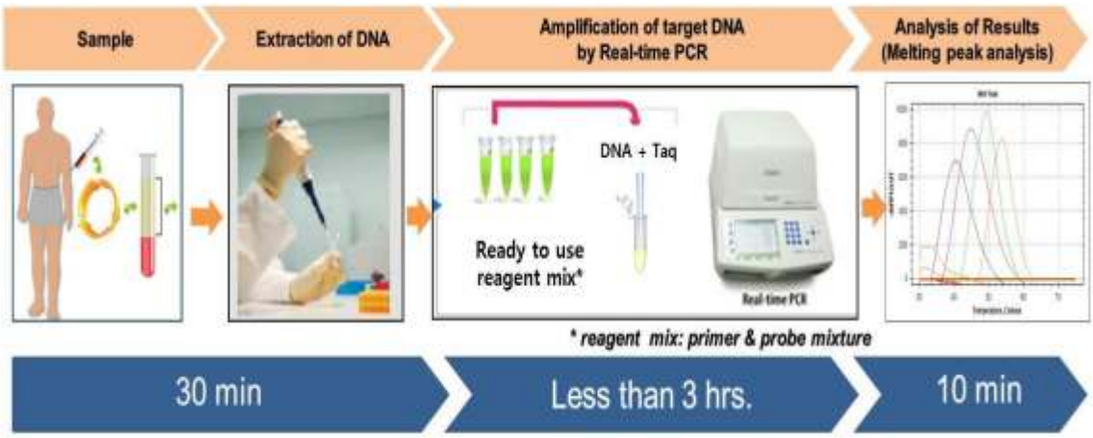


Figure 2. Workflow of the PANAMutyper™ R EGFR

1. Sample Preparation and Storage

Specimen collection and DNA extraction reagents are not included in the kit so they should be provided by the user.

1) Plasma, serum, formalin fixed paraffin embedded (FFPE) tissues, fresh tissues, biopsy tissues can be used as specimens.
2) Specimen transport: Use standard pathology methods to ensure specimen quality.
3) For DNA extraction, it is recommended to use the following DNA extraction kits.
   - Plasma and serum
     - ✓ QIAamp DSP Circulating NA Kit (Cat No. 61504, QIAGEN)
     - ✓ cobas® cfDNA Sample Preparation Kit (Cat No. 07247737190, Roche Molecular Systems)
     - ✓ TANBead® OptiPure cfDNA Reagent Tube(Cat No. 71CS46, Taiwan Advanced Nanotech Inc.)
   - Formalin-fixed paraffin-embedded (FFPE) tissue, fresh tissue, biopsy tissues and body fluid
     - ✓ High Pure PCR Template Preparation Kit (Cat No. 11 796 828 001, ROCHE)
     - ✓ Maxwell® 16 FFPE Plus LEV DNA Purification Kit (Cat No. AS1135, PROMEGA)
     - ✓ QIAamp DNA FFPE Tissue Kit (Cat No. 56404, QIAGEN)

4) Extracted DNA can be stored at 4℃ for up to 24 hours, or stored at -20℃ if it is over 24 hours after DNA extraction.

2. Preparation of the Real-Time PCR Mixture

Table 4. Set up reaction mixture per on reaction.

Components	Volume*
Each reagent mix (#1 ~ #6)	19 µl
Taq DNA polymerase (#7)	1 µl
Extracted DNA (25 ng total), Positive control or Negative control	5 µl
Total volume	25 µl

* Prepare one extra volume for each component for the pipette error.

1) Prepare each reagent mix after vortexing and spinning down at room temperature.
2) Prepare reaction sample (Extracted DNA) and control sample (NC, PC).
3) Prepare 6 PCR tubes for one set of DNA samples and label them as S1, S2, S3, S4, S5, and S6. Also, prepare another set of 6 tubes for PC and NC and label them as N1~N6 and P1~P6.
4) Load 19 µl of each reagent mix into the strip tubes or the PCR plate.
5) Load 1 µl of Taq DNA polymerase into the strip tubes or the PCR plate.
6) Load 5 µl of prepared DNA sample, PC and NC into each strip tubes or each well of the PCR plate to yield total 25 µl of final volume.
7) Seal the PCR plate tightly and spin down when all reagents are loaded. Otherwise, the PCR mixture can be evaporated and the result assessment for the sample is impossible.

3. Real-Time PCR reaction

Place the prepared strip tubes or the PCR plate on the block of your real-time PCR instrument. Please set the amplification condition according to the following Table 5.

Table 5. Real-time PCR protocol for PANAMutyper™ R EGFR

ONE CYCLE		
UDG incubation	50℃	2 min
Pre-denaturation	95℃	15 min
3-STEP CYCLING (15 CYCLES)		
Denaturation	95℃	30 sec
PNA clamping	70℃	20 sec
Annealing	63℃	1 min
3-STEP CYCLING (35 CYCLES)		
Denaturation	95℃	10 sec
Annealing & Detection*	53℃	20 sec
Extension	73℃	20 sec
MELTING CURVE ANALYSIS		
95℃		15 min
35℃		5 min
35℃ to 75℃ (increment 0.5℃)*		3 sec

* Select four fluorescent dyes (FAM, HEX, ROX, and Cy5 for CFX96 and FAM, VIC, ROX, and Cy5 for QuantStudio® 5) for all reaction wells(*).

4. PCR Result and Data Analysis

1) Set the baseline of G719X/S768I~L861Q melting peak according to the Table 6.

Table 6. The criteria of melting peak baseline

Reagent	Fluorescent Dye	Melting peak baseline	
		CFX96	QS5
G719X/S768I	FAM	100	3,000
	HEX (or VIC)	20	4,000
E19del/E20insA /EIC	HEX (or VIC)	100	4,000
	ROX	50	7,000
	Cy5	150	20,000
E20ins B	ROX	50	7,000
T790M	HEX (or VIC)	40	4,000
L858R	ROX	20	7,000
L861Q	ROX	100	7,000

2) C(t) determination mode

- For CFX96 device, set regression mode. And For QS5 device, set the baseline of Cy5 amplification plot(linear graph type) as 1/20 value of the max ΔRn.

3) Assess the result according to the fluorescent dye and melting temperature listed on Table 7. If the melting temperature for each fluorescent dye is in the proper range (See Table 7.), please assess the result as ltease as

Table 7. The criteria of the mutation detection according to the fluorescent dye and melting temperature

Bio-Rad CFX96					
Reagent	**Fluorescent Dye**	**Cut-off**	**Melting Temperature**	**Assessment**	
				Amino Acid Change	**Nucleotide Change**
G719X/ S768I	FAM	100	56.5℃~61.0℃	p.G719A	c.2156G>C
			44.5℃~49.0℃	p.G719S	c.2155G>A
			49.5℃~55.0℃	p.G719C	c.2155G>T
	HEX	20	58.5℃~62.0℃	p.S768I	c.2303G>T
E19del/E20ins A/EIC	HEX	100	59.5℃~68.0℃	E19del	
	ROX	50	61.5℃~70.0℃	E20ins	
	Cy5	150	56.0℃~64.0℃	Internal control	
E20ins B	ROX	50	61.5℃~70.0℃	E20ins	
T790M	HEX	40	58.0℃~63.0℃	p.T790M	c.2369C>T
L858R	ROX	20	55.0℃~58.0℃	p.L858R	c.2573T>G
			43.5℃~49.0℃	p.L858R	c.2573_2574 TG>GT
L861Q	ROX	100	48.0℃~54.5℃	p.L861Q	c.2582T>A

ABI QuantStudio® 5					
Reagent	Fluorescent Dye	Cut-off	Melting Temperature	Assessment	
				Amino Acid Change	Nucleotide Change
G719X/ S768I	FAM	3,000	55.5℃~62.3℃	p.G719A	c.2156G>C
			45.0℃~49.3℃	p.G719S	c.2155G>A
			49.4℃~55.4℃	p.G719C	c.2155G>T
	VIC	4,000	57.8℃~64.3℃	p.S768I	c.2303G>T
E19del/E20ins A/EIC	VIC	4,000	59.0℃~69.9℃	E19del	
	ROX	7,000	59.5℃~72.0℃	E20ins	
	Cy5	20,000	54.3℃~66.0℃	Internal control	
E20ins B	ROX	7,000	59.5℃~72.0℃	E20ins	
T790M	VIC	4,000	58.3℃~64.3℃	p.T790M	c.2369C>T
L858R	ROX	7,000	53.3℃~60.1℃	p.L858R	c.2573T>G
			42.7℃~51.1℃	p.L858R	c.2573_2574 TG>GT
L861Q	ROX	7,000	46.4℃~56.3℃	p.L861Q	c.2582T>A

* If the melting temperature for each fluorescence dye type is out of range (See Table 7.), please assess the result as negative.

** Tm values are rounded to second decimal places and applied to the criteria.

A. Positive control / Negative control

The results of the NC and PC should fall in the range given in Table 8. The assay should be repeated if the values are not in recommended range.

Table 8. The acceptable ranges of Ct and melting temperature for NC and PC.

Reagent	Fluorescent Dye	Analysis data	Equipment	Acceptable Ct range	
				Positive control	Negative control
E19del /E20ins A /EIC	Cy5	Ct	CFX96	10≤ Ct ≤18	10≤ Ct ≤18
			QS5	12≤ Ct ≤20	12≤ Ct ≤20
		Melting Temperature	CFX96	56.0℃~64.0℃	56.0℃~64.0℃
			QS5	54.3℃~66.0℃	54.3℃~66.0℃

Reagent	Acceptable Assessment		
	Positive control		Negative control
G719X/S768I	G719S	c.2155G>A	Wild
	S768I	c.2303G>T	Wild
E19del /E20ins A /EIC	E19del	E19del	Wild
	E20ins	E20ins	Wild
	Valid	-	Valid
E20ins B	E20ins	E20ins	Wild
T790M	T790M	c.2369C>T	Wild
L858R	L858R	c.2573T>G	Wild
L861Q	L861Q	c.2582T>A	Wild
Result	G719S, S768I, E19del, E20ins, T790M, L858R, L861Q		Wild

* Please refer to the Table 7. about melting temperature range of each mutation in Positive control.

B. DNA samples

a. Determine of the DNA sample's acceptability

i. Ct value of E19del/E20ins A/EIC should be bigger than 10 and less than 35.

ii. Ct value of E19del/E20ins A/EIC can serve as an internal control to indicate the purity and the concentration of DNA. Thus, the validity of the test can be decided by the Ct value and melting temperature of E19del/E20ins A/EIC as shown in Table 9.

Table 9. The acceptability of samples

Acceptability		E19del/E20ins A/EIC (Cy5)				Descriptions and recommendations
		Bio-Rad CFX96		ABI QuantStudio® 5		
		Ct value	Melting Temperature	Ct value	Melting Temperature	
Valid	Optimal	10< Ct <30	56.0℃~64.0℃	12< Ct <32	54.3℃~66.0℃	The amplification and the amount of DNA sample are optimal.
	Acceptable	30≤ Ct <35	56.0℃~64.0℃	32≤ Ct <35	54.3℃~66.0℃	The target gene was amplified with low efficiency. For more reliable result, it is suggested that repeat PCR reaction with a higher amount of DNA.
Invalid		Ct ≤ 10	-	Ct ≤ 12	-	Possibility of false positive is high. Repeat the PCR reaction with a lower amount of DNA.
		N/A	-	N/A	-	The PCR amplification failed. Please check the DNA amount and purity. It might be required to extract the DNA again.

b. Assess the result for each reagent along with the range of melting temperature and fluorescent dye as given in Table 7.

c. Assess the final result for 6 reagents included with E19del/E20ins A/EIC as given in Table 10.

Table 10. Final assessment

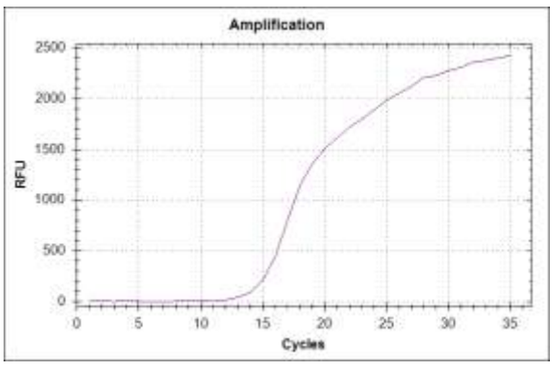
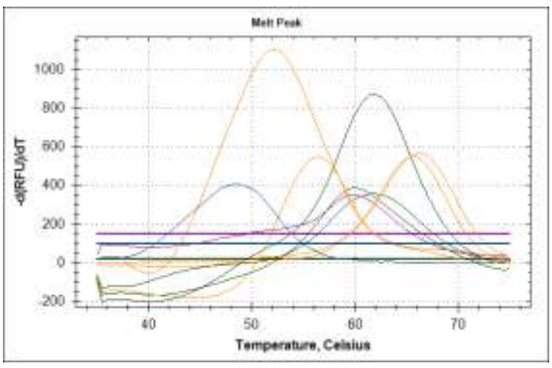
E19del/E20ins A/EIC	G719X/S768I, E19del/E20ins A/EIC, E20ins B, T790M, L858R and L861Q	Assessment
Valid	Mutant	Mutant ('Mutant' result is assessed by Table 7.)
Valid	Wild	Wild
Invalid	Mutant	Invalid
Invalid	Wild	Invalid

* If the assessed result for E19del/E20ins A/EIC is invalid for the clinical sample, the test result is 'invalid'. Please refer to recommendation for invalid sample as given in Table 9.

EXAMPLES OF ANALYSIS

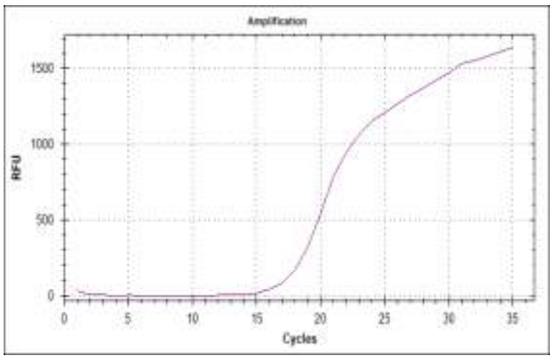
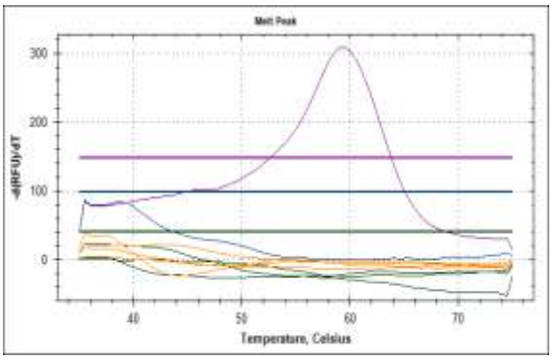
1. Bio-Rad CFX96

1) Profile of PC

Ct value (EIC)	Melting Temperature
	

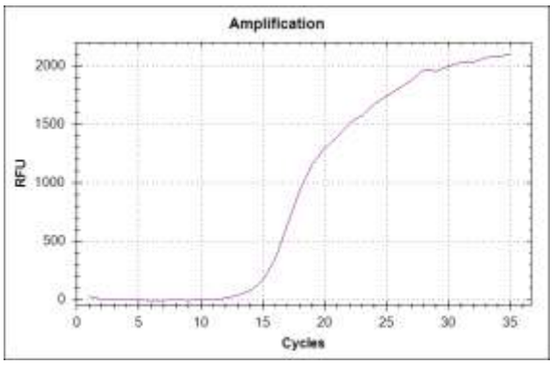
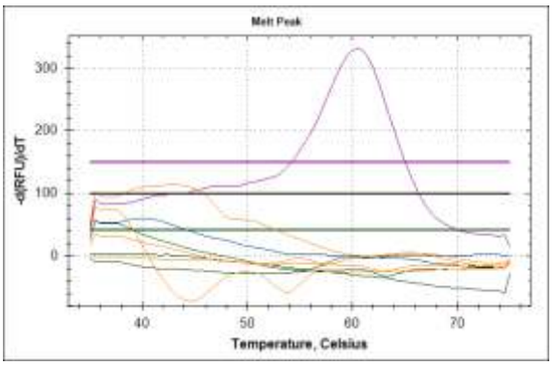
Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	48.5℃	FAM	44.5℃~49.0℃	Mutant	**G719S, S768I, E19del, E20ins, T790M, L858R, L861Q**
	-	HEX	60.0℃	HEX	58.5℃~62.0℃	Mutant	
② E19del /E20ins A/EIC	-	HEX	62.0℃	HEX	59.5℃~68.0℃	Mutant	
	-	ROX	65.5℃	ROX	61.5℃~70.0℃	Mutant	
	13.20	Cy5	60.0℃	Cy5	56.0℃~64.0℃	Valid	
③ E20ins B	-	ROX	66.0℃	ROX	61.5℃~70.0℃	Mutant	
④ T790M	-	HEX	62.0℃	HEX	58.0℃~63.0℃	Mutant	
⑤ L858R	-	ROX	56.5℃	ROX	55.0℃~58.0℃	Mutant	
⑥ L861Q	-	ROX	52.0℃	ROX	48.0℃~54.5℃	Mutant	

2) Profile of NC

Ct Value (EIC)	Melting Temperature
	

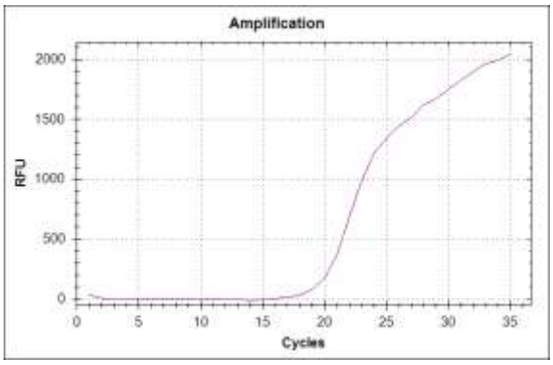
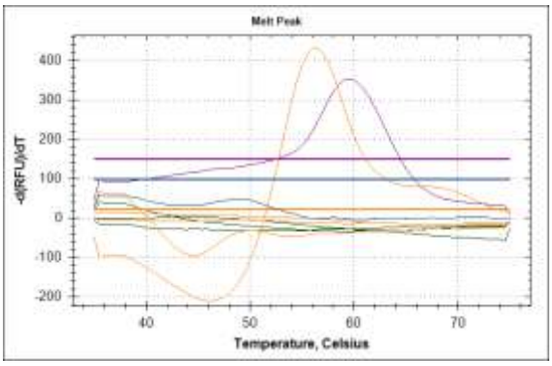
Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	-	-	-	Wild	Acceptable
	-	HEX	-	-	-	Wild	
② E19del /E20ins A/EIC	-	HEX	-	-	-	Wild	
	-	ROX	-	-	-	Wild	
	16.11	Cy5	59.5℃	Cy5	56.0℃~64.0℃	Valid	
③ E20ins B	-	ROX	-	-	-	Wild	
④ T790M	-	HEX	-	-	-	Wild	
⑤ L858R	-	ROX	-	-	-	Wild	
⑥ L861Q	-	ROX	-	-	-	Wild	

3) Profile of sample 1 (Wild)

Ct value (EIC)	Melting Temperature
	

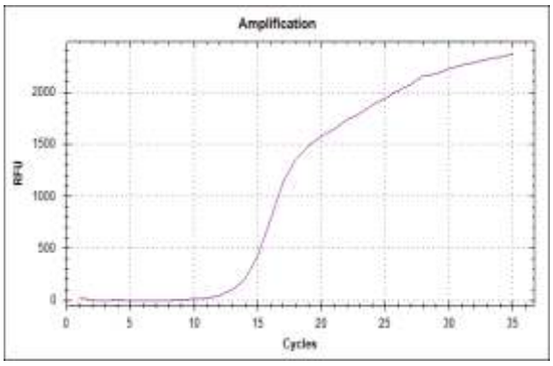
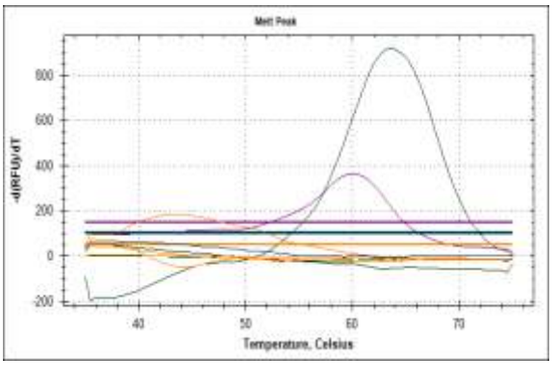
Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	-	-	-	Wild	Wild
	-	HEX	-	-	-	Wild	
② E19del /E20ins A/EIC	-	HEX	-	-	-	Wild	
	-	ROX	-	-	-	Wild	
	13.52	Cy5	60.5℃	Cy5	56.0℃~64.0℃	Valid	
③ E20ins B	-	ROX	-	-	-	Wild	
④ T790M	-	HEX	-	-	-	Wild	
⑤ L858R	-	ROX	-	-	-	Wild	
⑥ L861Q	-	ROX	-	-	-	Wild	

4) **Profile of sample 3 (L858R mutant)**

Ct value (EIC)	Melting Temperature
	

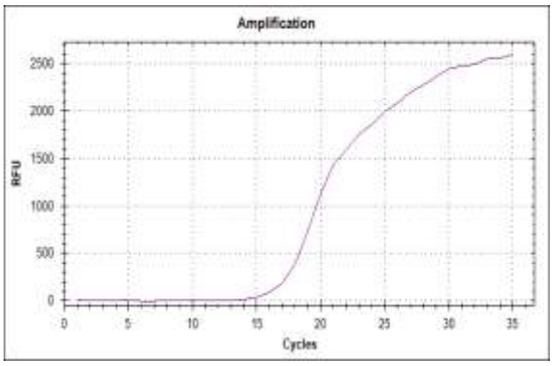
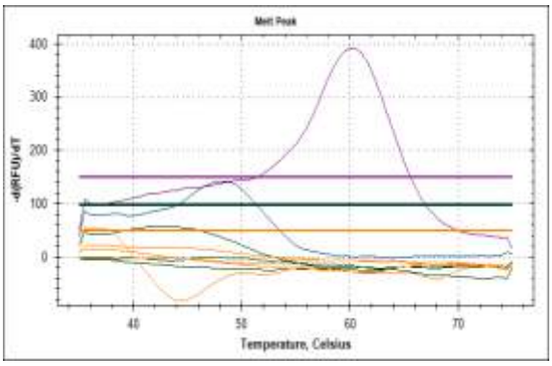
Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	-	-	-	Wild	L858R (Mutant)
	-	HEX	-	-	-	Wild	
② E19del /E20ins A/EIC	-	HEX	-	-	-	Wild	
	-	ROX	-	-	-	Wild	
	18.36	Cy5	59.5℃	Cy5	56.0℃~64.0℃	Valid	
③ E20ins B	-	ROX	-	-	-	Wild	
④ T790M	-	HEX	-	-	-	Wild	
⑤ L858R	-	ROX	56.5℃	ROX	55.0℃~58.0℃	Mutant	
⑥ L861Q	-	ROX	-	-	-	Wild	

5) Profile of sample 4 (E19del Mutant)

Ct value (EIC)	Melting Temperature
	

Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	-	-	-	Wild	E19del (Mutant)
	-	HEX	-	-	-	Wild	
② E19del /E20ins A/EIC	-	HEX	63.5℃	HEX	59.5℃~68.0℃	Mutant	
	-	ROX	-	-	-	Wild	
	12.36	Cy5	60.0℃	Cy5	56.0℃~64.0℃	Valid	
③ E20ins B	-	ROX	-	-	-	Wild	
④ T790M	-	HEX	-	-	-	Wild	
⑤ L858R	-	ROX	-	-	-	Wild	
⑥ L861Q	-	ROX	-	-	-	Wild	

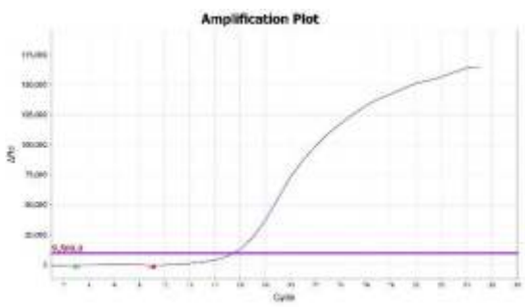
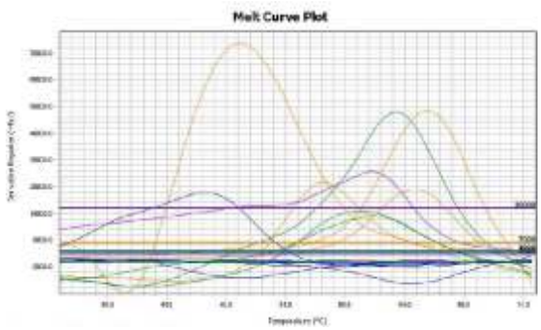
6) Profile of sample 5 (G719S mutant)

Ct value (EIC)	Melting Temperature
	

Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	48.0℃	FAM	44.5℃~49.0℃	Mutant	G719S (Mutant)
	-	HEX	-	-	-	Wild	
② E19del /E20ins A/EIC	-	HEX	-	-	-	Wild	
	-	ROX	-	-	-	Wild	
	12.58	Cy5	60.0℃	Cy5	56.0℃~64.0℃	Valid	
③ E20ins B	-	ROX				Wild	
④ T790M	-	HEX	-	-	-	Wild	
⑤ L858R	-	ROX	-	-	-	Wild	
⑥ L861Q	-	ROX	-	-	-	Wild	

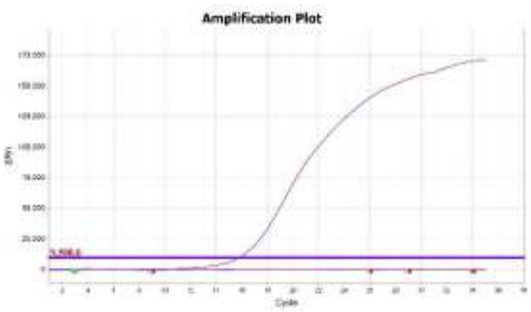
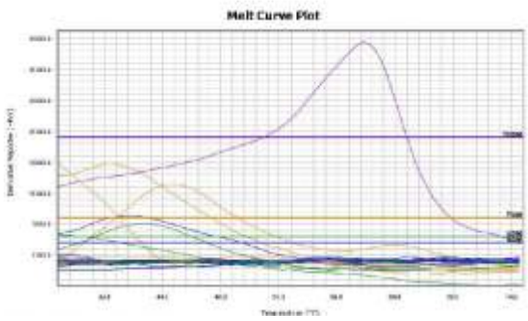
2. ABI QuantStudio® 5

1) Profile of PC

Ct value (EIC)	Melting Temperature
	

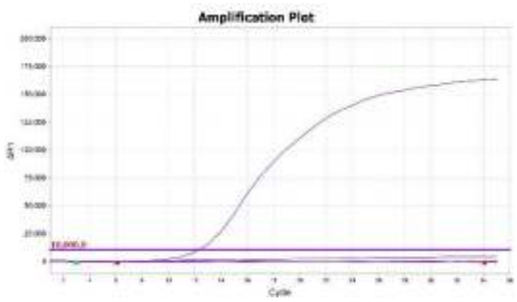
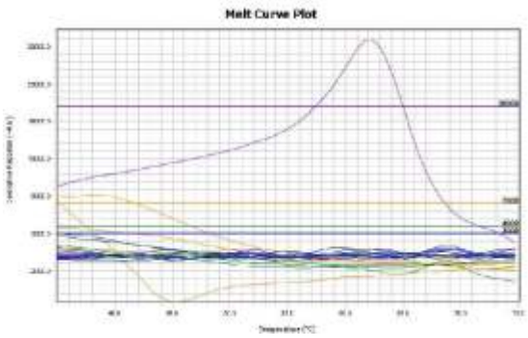
Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	47.2℃	FAM	45.0℃~49.3℃	Mutant	G719S, S768I, E19del, E20ins, T790M, L858R, L861Q
	-	VIC	60.5℃	VIC	57.8℃~64.3℃	Mutant	
② E19del /E20ins A/EIC	-	VIC	63.2℃	VIC	59.0℃~69.9℃	Mutant	
	-	ROX	61.3℃	ROX	59.5℃~72.0℃	Mutant	
	15.8	Cy5	60.0℃	Cy5	54.3℃~66.0℃	Valid	
③ E20ins B	-	ROX	65.0℃	ROX	59.5℃~72.0℃	Mutant	
④ T790M	-	VIC	61.5℃	VIC	58.3℃~64.3℃	Mutant	
⑤ L858R	-	ROX	57.2℃	ROX	42.7℃~51.1℃	Mutant	
⑥ L861Q	-	ROX	50.1℃	ROX	46.4℃~56.3℃	Mutant	

2) Profile of NC

Ct value (EIC)	Melting Temperature
	

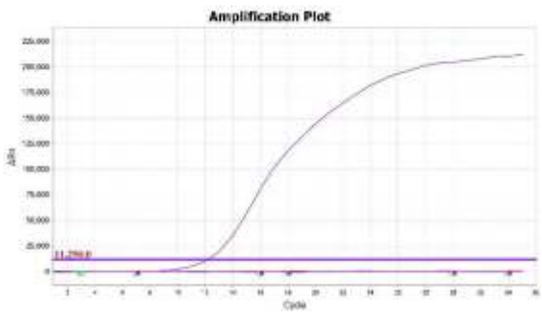
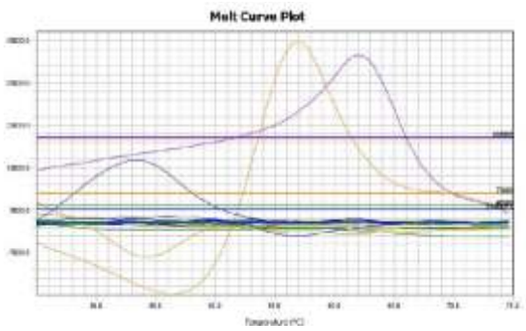
Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	-	-	-	Wild	Acceptable
	-	VIC	-	-	-	Wild	
② E19del /E20ins A/EIC	-	VIC	-	-	-	Wild	
	-	ROX	-	-	-	Wild	
	16.00	Cy5	61.5℃	Cy5	54.3℃~66.0℃	Valid	
③ E20ins B	-	ROX				Wild	
④ T790M	-	VIC	-	-	-	Wild	
⑤ L858R	-	ROX	-	-	-	Wild	
⑥ L861Q	-	ROX	-	-	-	Wild	

3) Profile of sample 1 (Wild)

Ct value (EIC)	Melting Temperature
	

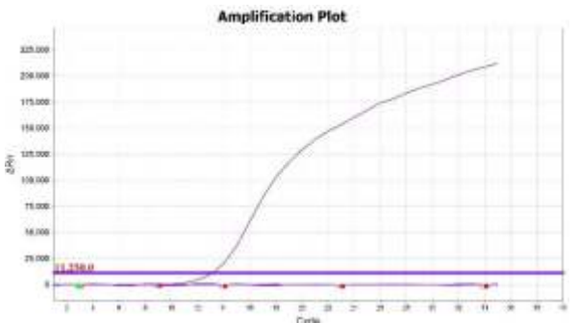
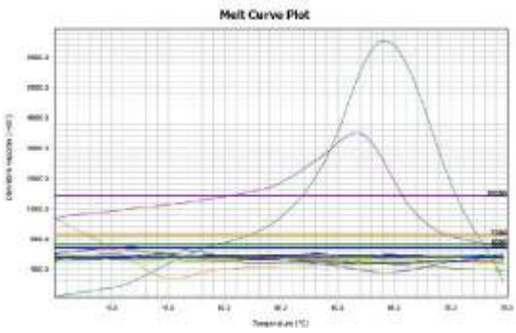
Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	-	-	-	Wild	**Wild**
	-	VIC	-	-	-	Wild	
② E19del /E20ins A/EIC	-	VIC	-	-	-	Wild	
	-	ROX	-	-	-	Wild	
	12.0	Cy5	62.0℃	Cy5	54.3℃~66.0℃	Valid	
③ E20ins B	-	ROX				Wild	
④ T790M	-	VIC	-	-	-	Wild	
⑤ L858R	-	ROX	-	-	-	Wild	
⑥ L861Q	-	ROX	-	-	-	Wild	

4) **Profile of sample 2 (L858R)**

Ct value (EIC)	Melting Temperature
	

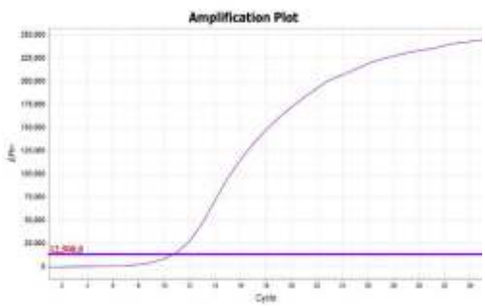
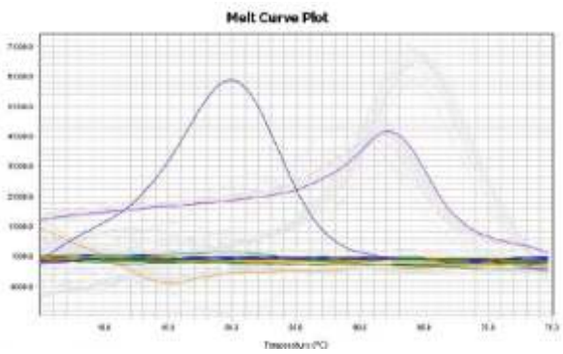
Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	-	-	-	Wild	L858R (Mutant)
	-	VIC	-	-	-	Wild	
② E19del /E20ins A/EIC	-	VIC	-	-	-	Wild	
	-	ROX	-	-	-	Wild	
	12.5	Cy5	59.5℃	Cy5	54.3℃~66.0℃	Valid	
③ E20ins B	-	ROX	-	-	-	Wild	
④ T790M	-	VIC	-	-	-	Wild	
⑤ L858R	-	ROX	57.5℃	ROX	53.3℃~60.1℃	Mutant	
⑥ L861Q	-	ROX	-	-	-	Wild	

5) Profile of sample 3 (E19del)

Ct value (EIC)	Melting Temperature
	

Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	-	-	-	Wild	E19del (Mutant)
	-	VIC	-	-	-	Wild	
② E19del /E20ins A/EIC	-	VIC	64.0℃	VIC	59.0℃~69.9℃	Mutant	
	-	ROX	-	-	-	Wild	
	13.7	Cy5	60.0℃	Cy5	54.3℃~66.0℃	Valid	
③ E20ins B	-	ROX				Wild	
④ T790M	-	VIC	-	-	-	Wild	
⑤ L858R	-	ROX	-	-	-	Wild	
⑥ L861Q	-	ROX	-	-	-	Wild	

6) **Profile of sample 4 (G719C)**

Ct value (EIC)	Melting Temperature
	

Reagent	Ct Value	Fluorescent Dye	Melting Temperature	Criteria of Table 7		Assessment	Result
				Fluorescent Dye	Melting Temperature		
① G719X /S768I	-	FAM	49.8℃	FAM	49.4℃~55.4℃	Mutant	**G719C (Mutant)**
	-	VIC	-	-	-	Wild	
② E19del /E20ins A/EIC	-	VIC	-	-	-	Wild	
	-	ROX	-	-	-	Wild	
	12.58	Cy5	60.0℃	Cy5	54.3℃~66.0℃	Valid	
③ E20ins B	-	ROX	-	-	-	Wild	
④ T790M	-	VIC	-	-	-	Wild	
⑤ L858R	-	ROX	-	-	-	Wild	
⑥ L861Q	-	ROX	-	-	-	Wild	

QUALITY CONTROL

Each lot of PANAMutyper™ R EGFR is tested against predetermined specifications to ensure consistent product quality in accordance with PANAGENE's ISO 9001 & 13485-certified Quality Management System.

PERFORMANCE TEST

1. Analytical Sensitivity

The analytical sensitivity of PANAMutyper™ R EGFR was determined using the standard DNAs that were isolated from EGFR mutant-type cell lines. The EGFR mutant-type DNAs were prepared to have 1, 0.5 and 0.1% or 1, 0.1. 0.01% of each EGFR mutation in a background of wild-type DNA. Three tests were performed with these 3 conditions of DNAs for 3 different batches of the kit. The results showed that this assay can detect each EGFR mutation at 0.1% or 0.5% DNA concentrations.

2. Analytical Specificity

The analytical specificity of PANAMutyper™ R EGFR was determined by testing the EGFR wild-type cell line DNA. Three tests were performed with the cell line DNA (25 and 50 ng). The results showed no detection of any EGFR mutation. The PANAMutyper™ R EGFR detected mutations from three EGFR mutant-type cell line DNAs from H1975 and PC9 cell lines without any cross-reactivity.

3. Reproducibility

The reproducibility test was conducted using the standard cell line DNAs that were mixture of mutant-type with 10^3, 10^2, and 10^1 copy in a background of wild-type. The tests were conducted by three laboratory professionals using the kits from three different batches in three days.

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3. Kobunai et al., The frequency of EGFR mutation detection in human colon carcinoma is influenced by the sensitivity of assay methodology : A comparison between direct sequencing and real-time PCR. Biochem Biophys Res Commun. 2010 Apr 23;395(1):158-62.
4. Chang et al., Fast simultaneous detection of K-RAS mutations in colorectal cancer. BMC Cancer. 2009 Jun 11;9:179.
5. Beau-Faller et al., Detection of K-Ras mutations in tumour samples of patients with non-small cell lung cancer using PNA-mediated PCR clamping. Br J Cancer. 2009 Mar 24;100(6):985-92.

EXPLANATION OF SYMBOLS ON THE LABEL

LOT	Batch Code		Use by (YYYY.MM.DD)
	Manufacturer	EC REP	EC Representative
IVD	In Vitro Diagnostic Medical Device	REF	Catalog number
	Temperature Limitation	CE	European conformity

CE

PANAGENE Inc.
54, Techno 10-ro, Yuseong-gu, Daejeon, 34027, Korea

EC REP **MT Promedt Consulting GmbH**
Altenhofstrasse 80, 66386 St. Ingbert, Germany