PANAGENE

PANA RealTyper HPV Kit

INTENDED USE

The PANA RealTyper™ HPV kit is an *in vitro* diagnostic reagent for genotyping of human papilloma virus (HPV) using peptide nucleic acid (PNA) probes. This kit is an amplified DNA test for the qualitative detection of a total of 40 HPV genotypes in a real-time PCR (polymerase chain reaction) system. This kit provides genotyping information of 20 high-risk and 2 low-risk types using melting temperature (Tm) analysis. Furthermore, this kit also detects 18 other genotypes (without genotyping) in DNA samples from clinical specimens.

Detectable genotypes by PANA RealTyper™ HPV kit

High-risk genotypes (20 genotypes)

- 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 70, 73, and 82

Low-risk genotypes (2 genotypes)

- 6 and 11

Other genotypes (detection only, 18 genotypes)

- 30, 32, 34, 40, 42, 43, 44, 54, 55, 61, 62, 67, 74, 81, 83, 84, 87, and 90

The PANA RealTyper™ HPV Kit 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.

PRINCIPLE AND OVERVIEW

The PANA RealTyper™ HPV Kit uses PNA probe-based fluorescence melting curve analysis technology in a real-time PCR system. Each genotype-specific PNA probe, which is conjugated with a fluorescent dye and a quencher, is used as a reporter in a real-time PCR reaction. These PNA probes are designed to hybridize only to their specific target sequence during the annealing step of PCR reaction. This specific binding is interfered by even a single mismatch between a PNA probe and a target sequence. Therefore, this PNA probe system has high specific target detection property and can be used to detect multiple targets in single PCR reaction tube.

Furthermore, PANA RealTyper™ HPV Kit implement melting curve analysis, and then HPV genotypes are determined by measuring a melting temperature. A total of 21 HPV genotypes-specific PNA probes have each unique Tm value. The combination of unique Tm and pre-determined fluorescent dye of PNA probe is used for genotyping amplified HPV DNA in the PCR reaction. This kit allows multiplex genotyping in a single PCR reaction. In addition, clinical sample with multiple infections of different HPV genotypes is also accurately determined using this kit.

The principle of the assay outlined in Figure 1. The kit is designed for detecting of a fragment in the L1 gene from HPV genome. Viral and human DNA is extracted from clinical specimens simultaneously. (Specimen collection and DNA extraction kits are not part of the kit.)

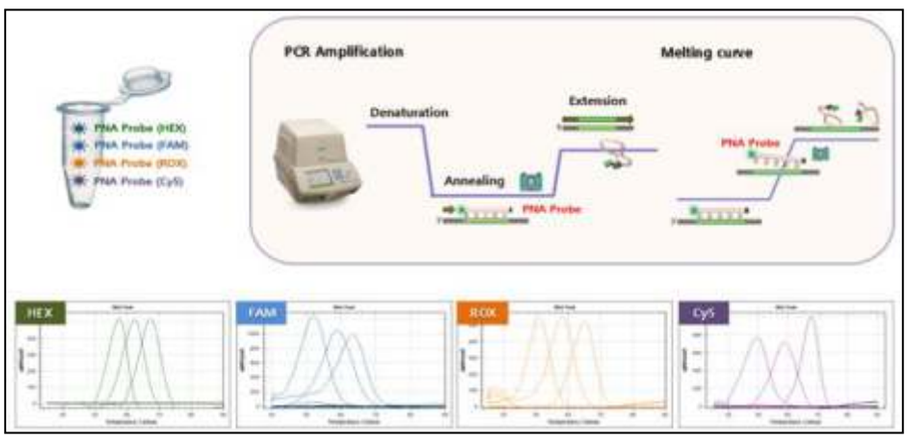

Figure 1. Principle of the PANA RealTyper™ HPV assay.

HPV Mixes #A and #B tubes contain a fluorescent dye [FAM, HEX (or VIC), ROX, or Cy5] and a quencher conjugated genotype specific PNA probes for 20 high-risk and 2 low-risk HPV genotypes. The HPV genotype can be identified by analyzing the unique Tm value. HPV Mix #O tube contains a fluorescent dye (FAM) and a quencher conjugated HPV target specific PNA probes. The Ct (cycle threshold) value of FAM signal will be used for detecting HPV DNA in the sample.

CONTACT INFORMATION

For any questions including technical support, please contact the distributors or the manufacturer.

Manufacturer: PANAGENE Inc.
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Email: info@panagene.com
Tel: +82 42 861 9296

EC Representative: MT Promedt Consulting GmbH
Altenhofstrasse 80,
66386 St. Ingbert, Germany
Email: info@mt-procons.com
Tel: +49 6894 581020

SAFETY INFORMATION

Material Safety Data Sheets (MSDS) are available on request.

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 1. Compatible real-time PCR instrument.

Company	Model
Bio-Rad	CFX 96
Applied Biosystems	QuantStudio® 5

WARNINGS AND PRECAUTIONS

Please read carefully this instruction and become familiar with all components of the kit prior to use.

PANA RealTyper™ HPV kit 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 6). 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 completed.

Do not use the reagents after their expiration date.

STORAGE CONDITION AND STABILITY

The PANA RealTyper™ HPV Kit 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 PANA RealTyper™ HPV kit should be stored immediately upon receipt at -15℃ to -20℃. When stored under this 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 48 samples can be tested using a kit.

Table 2. Reagents provided in the PANA RealTyper™ HPV kit.

No.	Name of content	Description	Volume	Label & color of cap
1	HPV Mix #A	HPV PNA probes and primers	1000 l	**HPV #A**
2	HPV Mix #B	HPV PNA probes and primers	1000 l	**HPV #B**
3	HPV Mix #O	HPV PNA probes, HBB PNA probe, and primers	1000 l	**HPV #O**
4	Taq DNA polymerase	Taq DNA polymerase	170 l	**Taq**
5	Positive Control (PC)	Positive Control	100 l	**P.C**
6	Negative Control (NC)	Negative Control	100 l	**N.C**

PROCEDURES

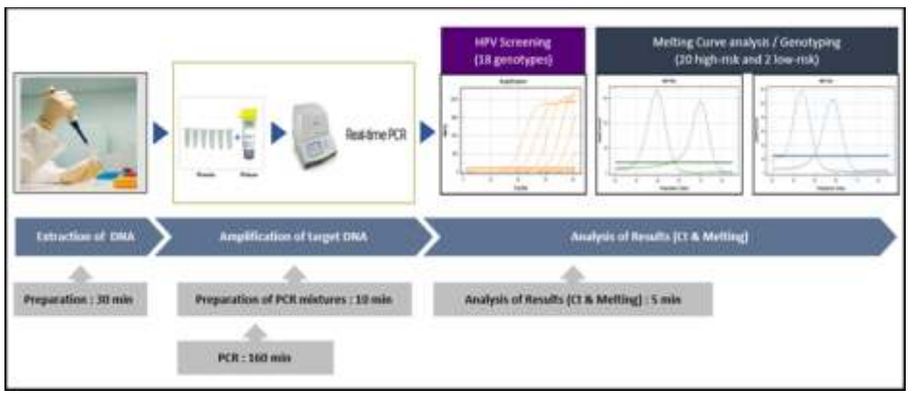


Figure 2. Workflow of the PANA RealTyper™ HPV kit.

1. **Sample preparation and storage** (not provided in this kit)

1) A Swab Sampler should be used for the collection of cervical specimen.

2) Ideally, cervical specimens should be processed on the same day when they are collected. Alternatively, they can be stored at 4℃ for maximum seven days.

3) DNA can be isolated using one of recommended kits in Table 3.

Table 3. The list of recommended DNA isolation kit.

Model	Company	Catalog number
Exgene Blood SV	GeneAll (South Korea)	104-152
QIAamp DNA Blood Mini Kit	Qiagen (USA)	51104
Insta Gene Matrix	Bio-Rad (USA)	732-6030
MaxWell® 16 Viral Total Nucleic Acid Purification Kit	Promega (USA)	AS1150

4) Extracted DNA can be stored at 4℃ for up to seven days or at -20℃ for long term storage.

2. Preparation of the real-time PCR reaction mixture

Table 4. Set up reaction mixture per one reaction.

Reagent	**Volume***
HPV mix (HPV Mix #A, #B, or #O, respectively)	19 1
Taq DNA polymerase	1 1
Extracted DNA, PC, or NC	5 1
Total volume	25 1

* Prepare one extra volume for each component to compensate pipette error.

1) Prepare three reagent mixes (HPV Mix #A, #B, and #O) after thaw, vortex and spin down at room temperature.
2) Prepare test sample (extracted DNA) and control samples (NC and PC).
3) Prepare 3 PCR tubes (or wells) for one DNA sample to be tested. Label them as A1, B1, and O1, if it is necessary.
4) Load 19 µl of each reagent mix (HPV Mix #A, #B, or #O) into a strip tube or the PCR plate. For example, A1 tube (or well) will contain HPV Mix #A, B1 tube (or well) will contain HPV Mix #B, O1 tube (or well) will contain HPV Mix #O.
5) Add 1 µl of Taq DNA polymerase to the strip tubes or the PCR plate.
6) Add 5 µl of prepared test sample into each strip tubes or each well of the PCR plate to yield a total 25 l of final volume.
7) One set of PC and NC for each HPV Mix should be included in each run. Add 5 µl of PC or NC into strip tubes or wells of the PCR plate to yield a total 25 l of final volume.
8) Seal the PCR plate tightly and spin down. Otherwise, the PCR mixture can be evaporated and the result of the test may not accurate.

3. Real-time PCR reaction

1) Place the prepared strip tubes or the PCR plate on the block of a real-time PCR instrument.

2) Please set the PCR protocol according to following Table 5.

3) Select four fluorescent dyes (FAM, HEX(or VIC), ROX, and Cy5) for all reaction wells (*).

Table 5. Real-time PCR protocol for PANA RealTyper™ HPV kit.

ONE CYCLE		
Incubation	50℃	2 min
Taq activation	95℃	15 min
3-STEP CYCLING (45 CYCLES)		
Denaturation	95℃	15 sec
Annealing and Detection*	55℃	45 sec
Extension	72℃	15 sec
MELTING CURVE ANALYSIS		
95℃		5 min
35℃		5 min
35℃ to 80℃ (increment 0.5℃)*		5 sec

4. PCR result and data analysis

1) Set the baseline threshold of melting peak analysis according to each tube and dye, respectively (Table 6).

Table 6. The criteria of melting peak baseline

HPV Mix	Fluorescent Dye	Baseline threshold values	
		Bio-Rad CFX96	ABI QS5
HPV Mix #A	FAM	100	10,000
	HEX(or VIC)	50	7,000
	ROX	50	10,000
	Cy5	150	15,000
HPV Mix #B	FAM	100	10,000
	HEX(or VIC)	50	7,000
	ROX	50	10,000
	Cy5	100	15,000
HPV Mix #O	FAM	Regression	80,000
	Cy5	Regression	10,000

2) Select regression mode for Ct determination.

3) Assess the results according to the fluorescent dyes, HPV mixes and melting temperatures listed on Tables 7 and 8.

A. Negative control and Positive control

The Ct values and melting temperatures of the NC and PC must fall into the ranges that given in Table 7. The assay must be repeated if the values are not in these recommended ranges.

Table 7. The Acceptable ranges of Ct and melting temperature for NC and PC.

<Bio-Rad CFX96>

	Negative Control		
Fluorescent Dye	**HPV Mix #A (Tm)**	**HPV Mix #B (Tm)**	**HPV Mix #O (Ct)**
FAM	-	-	-
HEX (or VIC)	-	-	-
ROX	-	-	-
Cy5	-	-	18.0-35.0

	Positive Control		
Fluorescent Dye	**HPV Mix #A (Tm)**	**HPV Mix #B (Tm)**	**HPV Mix #O (Ct)**
FAM	55.0-60.0	60.0-65.0	18.0-35.0
HEX (or VIC)	45.0-50.0	47.0-51.0	-
ROX	56.0-61.0	56.0-65.0	-
Cy5	50.0-54.0	51.0-55.0	18.0-35.0

<ABI QS5>

	Negative Control		
Fluorescent Dye	**HPV Mix #A (Tm)**	**HPV Mix #B (Tm)**	**HPV Mix #O (Ct)**
FAM	-	-	-
HEX (or VIC)	-	-	-
ROX	-	-	-
Cy5	-	-	18.0-35.0

	Positive Control		
Fluorescent Dye	**HPV Mix #A (Tm)**	**HPV Mix #B (Tm)**	**HPV Mix #O (Ct)**
FAM	55.0-60.0	60.0-70.0	18.0-35.0
HEX (or VIC)	45.0-50.0	47.0-51.0	-
ROX	56.0-61.0	56.0-65.0	-
Cy5	48.5-54.5	50.5-56.5	18.0-35.0

B. HPV genotyping and screening

If the Tm or Ct value of each fluorescent dye in each Mix is in the criteria range (Table 8), please assess the result as 'Positive'. If the Tm or Ct value of each fluorescent dye is out of the criteria range, please assess the result as 'Negative'.

Table 8. Criteria of HPV genotyping according to reporter dye and melting temperature.

Bio-Rad CFX96								
HPV Mix #A			HPV Mix #B			HPV Mix #O		
Genotype	Reporter Dye	Tm (℃)	Genotype	Reporter Dye	Tm (℃)	Target	Reporter Dye	Ct
HPV 33	FAM	45.0-49.5	HPV 53	FAM	48.0-54.0	HPV: 30, 32, 34, 40, 42, 43, 44, 54, 55, 61, 62, 67, 74, 81, 83, 84, 87, and 90	FAM	10≤Ct≤40
HPV 11		55.0-60.0	HPV 35		60.0-65.0			
HPV 58		60.5-67.0	HPV 39	HEX	47.0-51.0			
HPV 16	HEX	45.0-50.0	HPV 59		52.0-55.0			
HPV 73		61.0-65.0	HPV 31		58.0-64.0			
HPV 45	ROX	43.0-49.0	HPV 68	ROX	39.0-49.5			
HPV 18		56.0-61.0	HPV 70		50.0-55.0			
HPV 69		66.0-70.0	HPV 56		56.0-65.0			
HPV 26	Cy5	42.0-46.0	HPV 51	Cy5	43.0-47.0	Internal control (HBB)	Cy5	10≤Ct≤ 40
HPV 6		50.0-54.0	HPV 66		51.0-55.0			
HPV 52		59.5-64.0	HPV 82		58.0-62.0			

ABI QS5								
HPV Mix #A			HPV Mix #B			HPV Mix #O		
Genotype	Reporter Dye	Tm (℃)	Genotype	Reporter Dye	Tm (℃)	Target	Reporter Dye	Ct
HPV 33	FAM	44.5–50.0	HPV 53	FAM	48.0–54.0	HPV: 30, 32, 34, 40, 42, 43, 44, 54, 55, 61, 62, 67, 74, 81, 83, 84, 87, and 90	FAM	10≤Ct≤40
HPV 11		55.0–60.0	HPV 35		60.0–70.0			
HPV 58		61.5–69.5	HPV 39	VIC	47.0–51.0			
HPV 16	VIC	45.0–50.0	HPV 59		52.0–55.0			
HPV 73		61.0–65.0	HPV 31		58.0–64.0			
HPV 45	ROX	44.0–50.0	HPV 68	ROX	39.0–49.5			
HPV 18		56.0–61.0	HPV 70		50.0–55.0			
HPV 69		67.0–71.5	HPV 56		56.0–65.0			
HPV 26	Cy5	42.0–46.0	HPV 51	Cy5	43.0–47.0	Internal control (HBB)	Cy5	10≤Ct≤ 40
HPV 6		48.5–54.5	HPV 66		50.5–56.5			
HPV 52		59.5–64.0	HPV 82		58.5–63.0			

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

C. Interpretation of results

Test results are interpreted as shown in Table 9.

Table 9. Results interpretation of PANA RealTyper™ HPV Kit test.

Internal control (HBB, Ct value)	HPV Mix #A, #B, or #O	Interpretation
Valid (10≤Ct≤ 40)	Positive	HPV detected*
Valid (10≤Ct≤ 40)	Negative	HPV Not detected†
Invalid (Ct> 40)	Positive	HPV detected*
Invalid (Ct> 40)	Negative	Invalid#

HPV detected (*)

Positive results in HPV Mixes indicate presence of HPV DNA in the clinical sample even internal control shows negative result. The HPV genotype should be identified from criteria in Table 8.

HPV not detected: tested 40 HPV genotypes are not detected (†)

Positive result of internal control (IC) and negative results in all of three HPV Mixes indicate no detection of HPV DNA in the clinical sample. However, it cannot rule out the possibility that non-targeted HPV genotypes can be presence in the tested clinical sample. “HPV Not Detected” result does not preclude the presence of HPV in the clinical sample because results depend on adequate specimen integrity, absence of inhibitors, and sufficient DNA to be detected.

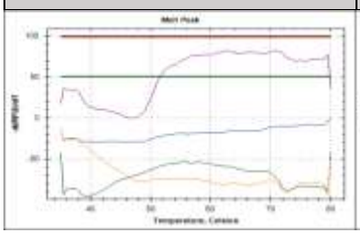
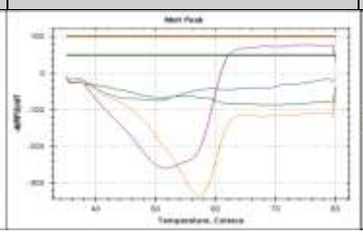
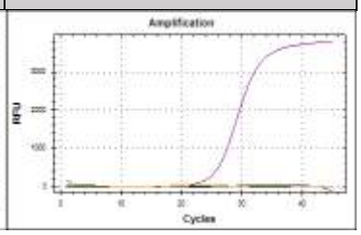
Invalid (#)

A Clinical test is invalid when IC and all of three HPV Mixes show negative results. It may denote that the amplification of HPV DNA sample is failed or the amount of isolated DNA is not sufficient. Please repeat the entire test procedure for that clinical sample, starting with DNA isolation.

EXAMPLES OF ANALYSIS

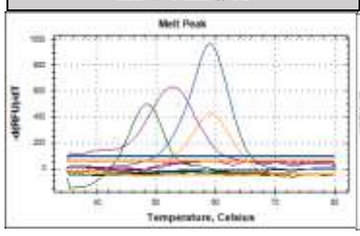
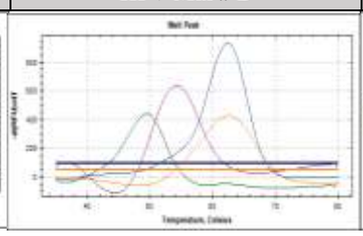
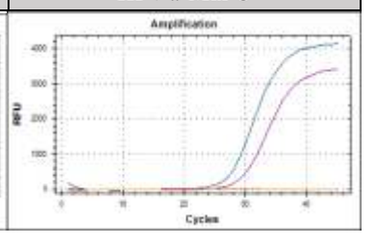
1. Bio-Rad CFX96

1) Negative Control (NC)

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

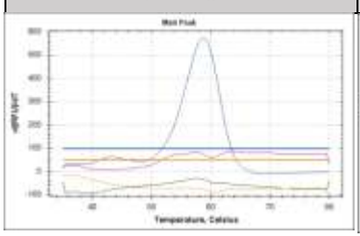
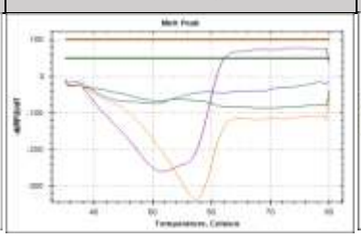
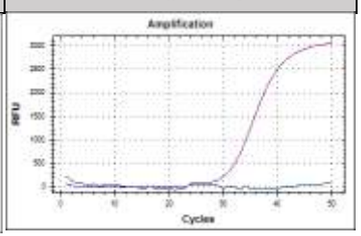
Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	All	-	Negative	**Acceptable**
HPV Mix #B	All	-	Negative	
HPV Mix #O	FAM	-	Negative	
	Cy5	24.77 (Ct)	Valid	

2) Positive Control (PC)

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

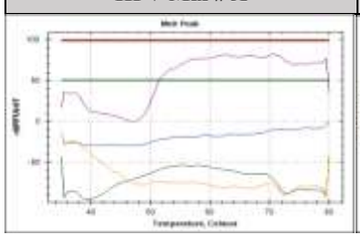
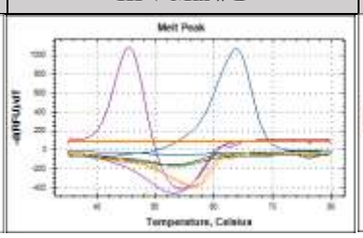
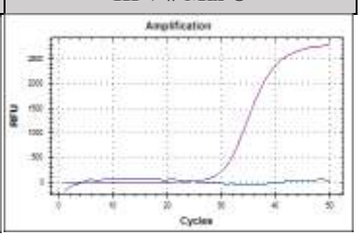
Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	FAM	59℃	Positive	**Acceptable**
	HEX	48.5℃		
	ROX	59℃		
	Cy5	52.5℃		
HPV Mix #B	FAM	62.5℃	Positive	
	HEX,	49.5℃		
	ROX	62.5℃		
	Cy5	54.5℃		
HPV Mix #O	FAM	25.77 (Ct)	Positive	
	Cy5	26.72 (Ct)	Valid	

3) Sample 1

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

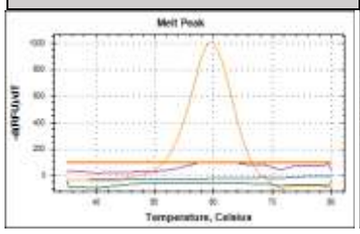
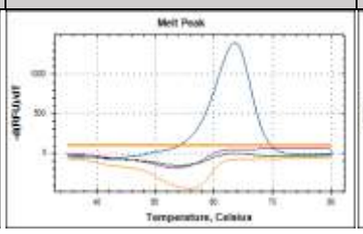
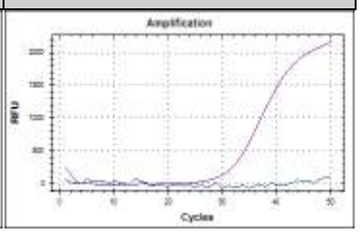
Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	FAM	59℃	Positive	**Type 11**
HPV Mix #B	All	-	Negative	
HPV Mix #O	FAM	-	Negative	
	Cy5	31.14 (Ct)	Valid	

4) Sample 2

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

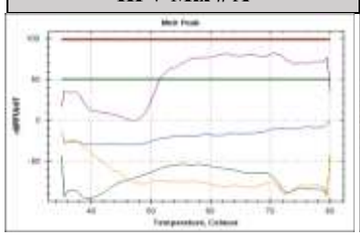
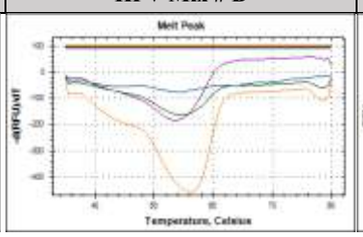
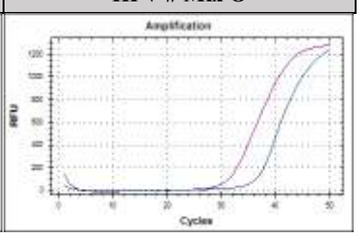
Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	All	-	Negative	**Type 51 & 35**
HPV Mix #B	FAM	64℃	Positive	
	Cy5	45.5℃		
HPV Mix #O	FAM	-	Negative	
	Cy5	30.62 (Ct)	Valid	

5) Sample 3

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

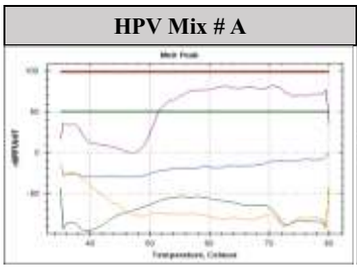
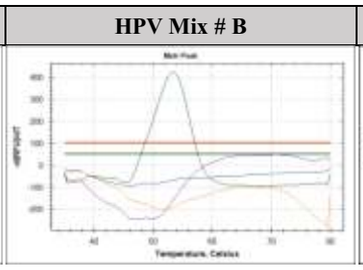
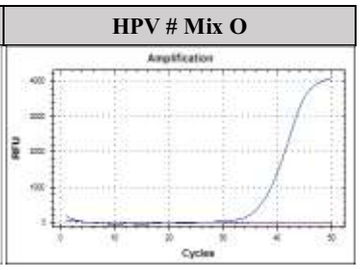
Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	ROX	59.5℃	Positive	**Type 18 & 35**
HPV Mix #B	FAM	63.5℃	Positive	
HPV Mix #O	FAM	-	Negative	
	Cy5	32.68 (Ct)	Valid	

6) Sample 4

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

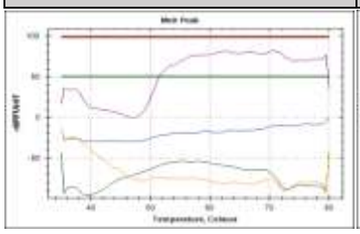
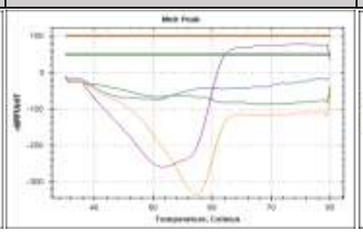
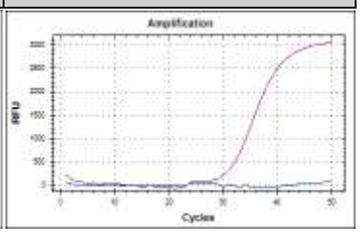
Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	All	-	Negative	**Other types**
HPV Mix #B	All	-	Negative	
HPV Mix #O	FAM	32.01 (Ct)	Positive	
	Cy5	35.99 (Ct)	Valid	

7) Sample 5

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	All	-	Negative	**Type 59 & other types**
HPV Mix #B	HEX	53.5℃	Positive	
HPV Mix #O	FAM	35.79 (Ct)	Positive	
	Cy5	-	Invalid	

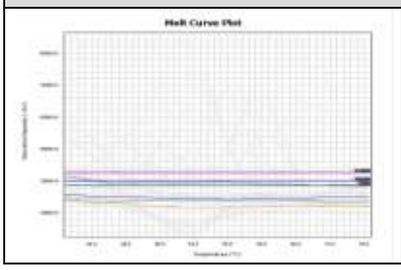
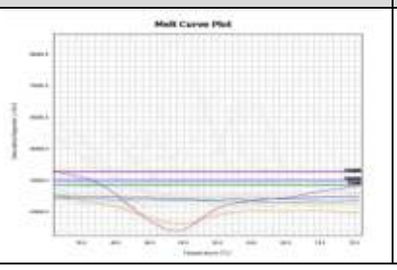
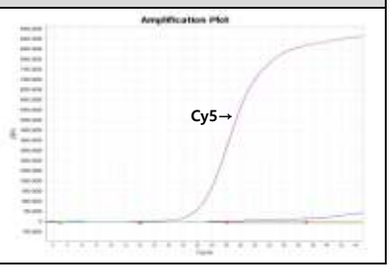
8) Sample 6

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	All	-	Negative	**HPV Not detected**
HPV Mix #B	All	-	Negative	
HPV Mix #O	FAM	-	Negative	
	Cy5	31.24 (Ct)	Valid	

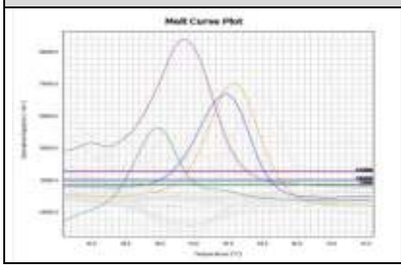
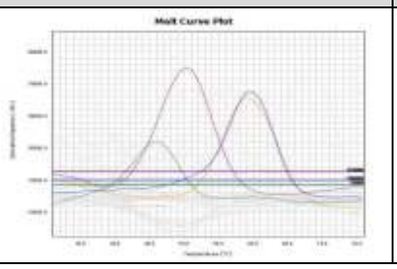
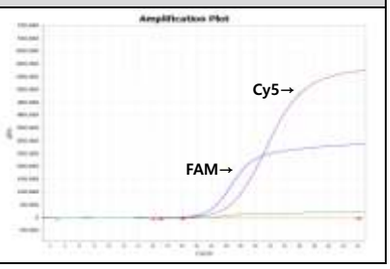
2. ABI QS5

1) Negative Control (NC)

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

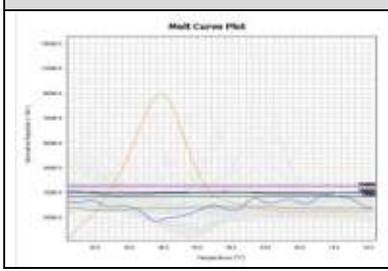
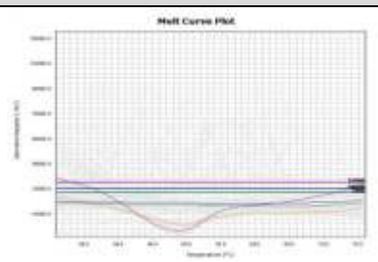
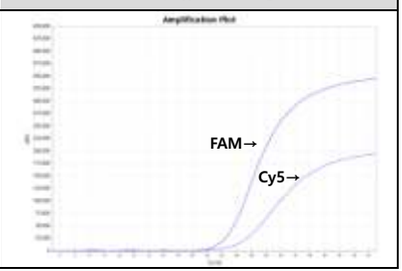
Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	All	-	Negative	**Valid**
HPV Mix #B	All	-	Negative	
HPV Mix #O	FAM	-	Negative	
	Cy5	19.2 (Ct)	Valid	

2) Postive Control (PC)

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

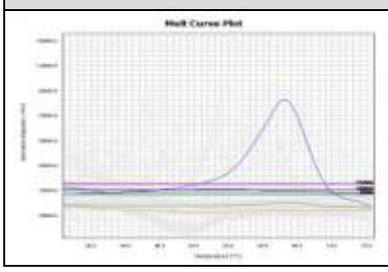
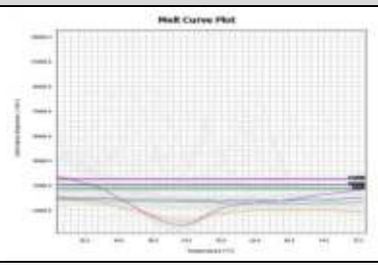
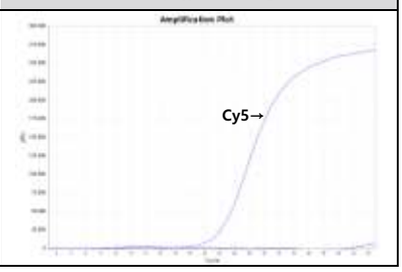
Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	FAM	58.6℃	Positive	**Valid**
	VIC	48.6℃		
	ROX	59.8℃		
	Cy5	52.6℃		
HPV Mix #B	FAM	63.5℃	Positive	
	VIC	49.9℃		
	ROX	63.0℃		
	Cy5	54.4℃		
HPV Mix #O	FAM	25.6 (Ct)	Positive	
	Cy5	23.6 (Ct)	Valid	

3) Sample 1

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

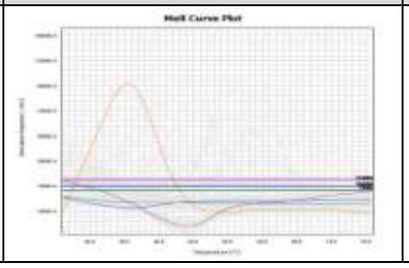
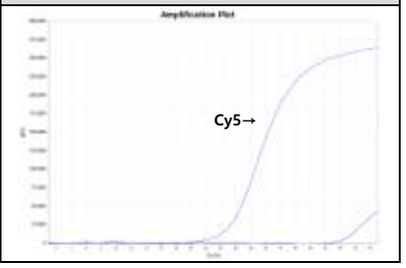
Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	ROX	48.6℃	Positive	**Type 45, Other**
HPV Mix #B	All	-	Negative	
HPV Mix #O	FAM	22.9 (Ct)	Positive	
	Cy5	25.1 (Ct)	Valid	

4) Sample 2

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

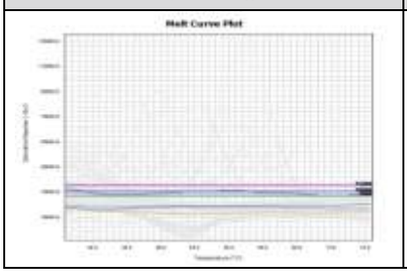
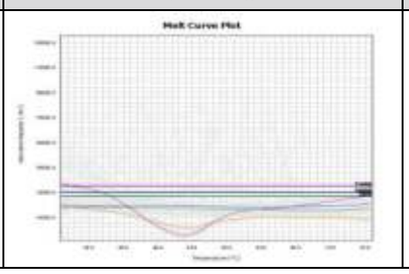
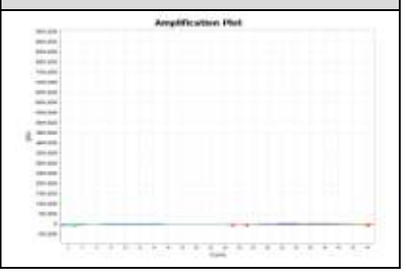
Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	FAM	67.0℃	Positive	**Type 58**
HPV Mix #B	All	-	Negative	
HPV Mix #O	FAM	-	Negative	
	Cy5	24.7 (Ct)	Valid	

5) Sample 3

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	All	-	Negative	**HPV 68**
HPV Mix #B	ROX	44.4℃	Positive	
HPV Mix #O	FAM	-	Negative	
	Cy5	24.7 (Ct)	Valid	

6) Sample 4

HPV Mix # A	HPV Mix # B	HPV # Mix O
		

Reagent	Fluorescent Dye	Melting temperature or Ct	Assessment	Result
HPV Mix #A	All	-	Negative	**Invalid**
HPV Mix #B	All	-	Negative	
HPV Mix #O	FAM	-	Negative	
	Cy5	-	Invalid	

QUALITY CONTROL

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

PERFORMANCE TESTS

1. Analytical Sensitivity

The detection limit was established using standard materials (plasmids containing the sequence from the L1 region of HPV genome). Measured lowest concentrations as limit of detections were summarized in Table 10.

Table 10. Limit of detection for PANA RealTyper™ HPV Kit.

LoD (copies)	HPV genotypes	No. of genotypes
5×10^1	6, 11, 16, 35, 53, 58, 59, 62, 70 and 83	10
5×10^2	18, 30, 31, 33, 34, 39, 40, 42, 45, 52, 54, 56, 66, 67, 68, 69, 74, 81, 82, 84 and 90	21
5×10^3	26, 32, 44, 51, 55, 61, 73 and 87	8
5×10^4	43	1

2. Analytical Specificity

1) Cross-reactivity to pathogenic microorganisms related to other diseases

The test was conducted using three batches of kits and DNA samples that were isolated from 14 microorganisms causing various infectious diseases in human. The kit showed no cross-reactivity with any below tested pathogenic microorganisms.

Bacteria	Bacteria	Bacteria
Veilloneella sp.	*Ureaplasma urealyticum*	*Staphylococus epidermidis*
Klebsiella oxytoca	*Mycoplasma genitalium*	*Haemophilus ducreyi*
Pseudomonas aeruginosa	*Streptococcus* sp.	
Gardnerella vaginalis	*Treponema phagedenis*	
Enterococcus faecium	*Neisseria gonorrhoeae*	Yeast
Proteus mirabilis	*Staphylococus epidermidis*	*Candida albicans*

2) Cross-reactivity to non-specific HPV genotypes

Each PNA probe tested cross-reactivity using the 40 different standard materials of HPV genotypes. The tests were conducted in triplicate using the kits from three different batches. Each individual PNA probe showed no cross-reactivity with standard materials except own target.

3. Reproducibility

The reproducibility test was conducted using the standard materials of 40 HPV genotypes that were diluted at the concentration between 5×10^{7} and 5×10^{1} copies. The tests were conducted in triplicate using the kits from three different batches in three days. All the results showed close to 5% CV (coefficient of variation).

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1. Choi, J. J. *et al.* Peptide nucleic acid-based array for detecting and genotyping human papillomaviruses. *J Clin Microbiol* 2009; 1785-1790.
2. Gerard, J. *et al.* Correlation of Pap smear, cervical biopsy, and clinical follow-up with an HPV typing microarray system. *Diagn Mol Pathol* 2008; 17:107-111.
3. Debbie Saslow *et al.* American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the Prevention Early Detection of cancer. *Am J Olin Paihol* 2012; 137 : 516-542
4. Abreu, *et al.* A review of methods for detect human Papillomavirus infection. *Virology Journal* 2012, 9:262

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	Catalogue 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