PANA RealTyper HPV Screening Kit

PANAGENE

INTENDED USE

The PANA RealTyper™ HPV Screening Kit is an *in vitro* diagnostic reagent for detecting of human papilloma virus (HPV) from cervical swab. This kit is an amplified DNA test for the qualitative detection of a total of 14 high-risk (HR) HPV types and 2 low-risk (LR) HPV types in a real -time PCR (polymerase chain reaction) system. This kit specially identifies 2 HR types such as 16 and 18 and 2 LR types such as 6 and 11 while concurrently detecting the other HR HPV types that includes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

The PANA RealTyper™ HPV Screening Kit indicated:

To screen patients 21 years and older with ASC-US (atypical squamous cells of undetermined significance) cervical cytology test results to determine the need for referral to colposcopy.

To be used in patients 21 years and older with ASC-US cervical cytology results, to detect high-risk HPV genotypes 16 and 18. This information, together with the physician's assessment of screening history, other risk factors, and professional guidelines, may be used to guide patient management. The results of this test are not intended to prevent women from proceeding to colposcopy.

In women 30 years and older, the PANA RealTyper™ HPV Screening Kit can be used with cervical cytology to adjunctively screen to detect high risk HPV types. This information, together with the physician's assessment of screening history, other risk factors, and professional guidelines, may be used to guide patient management.

In women 30 years and older, the PANA RealTyper™ HPV Screening Kit can be used to detect HPV genotypes 16 and 18. This information, together with the physician's assessment of screening history, other risk factors, and professional guidelines, may be used to guide patient management.

In women 25 years and older, the PANA RealTyper™ HPV Screening Kit can be used as a first-line primary cervical cancer screening test to detect high risk HPV, including genotyping for 16 and 18. Women who test negative for high risk HPV types by the PANA RealTyper™ HPV Screening Kit should be followed up in accordance with the physician's assessment of screening and medical history, other risk factors, and professional guidelines. Women who test positive for HPV genotypes 16 and/or 18 by the PANA RealTyper™ HPV Screening Kit should be referred to colposcopy. Women who test high risk HPV positive and 16/18 negative by the PANA RealTyper™ HPV Screening Kit (12 other HR HPV positive) should be evaluated by cervical cytology to determine the need for referral to colposcopy.

The PANA RealTyper™ HPV Screening Kit is a CE marked diagnostic device in accordance with the European Union *in vitro* Diagnostic Medical Device Directive 98/79/EC.

PANA RealTyper HPV Screening Kit

PRINCIPLE AND OVERVIEW

PANA RealTyper™ HPV Screening K it uses PNA probe-based fluorescence melting curve analysis technology in a real-time PCR system. Each 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 hybridizeonly to their specific target sequence during the annealing step of PCR reaction. This specific binding is interfered by even a single mismatchbetween 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.

Furthermore, PANA RealTyper™ HPV Screening Kit implements melting curve analysis,and then HPV types are detected by measuring a melting temperature. The specific PNA probes have each unique Tm value. The combination of unique Tm and pre-determined fluorescent dye of PNA probe is used for detecting amplified HPV DNA in the PCR reaction. This kit allows multiplex genotyping in a single PCR reaction.

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

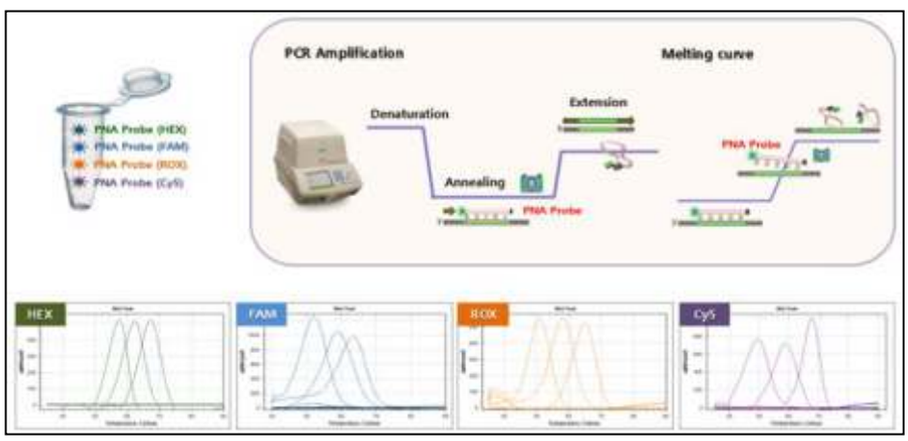


Figure 1. Principle of the PANA RealTyper™ HPV Screening Kit

HPVS Mix tube contain a fluorescent dye (FAM, HEX (or VIC), ROX,or Cy5) and a quencher conjugated specific PNA probes for 16 HPV genotypes and H BB. The HPVgenotype can be identified or detected by analyzing the unique Tm value.

EQUIPMENT AND MATERIA LS 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

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

Table 1. Compatible real time PCR instruments and plastic consumables

Company	Model	Consumables
Bio-Rad	CFX96	White PCR plate (Catalog No. BRMLL-9651) Adhesive seals (Catalog No. MSB-1001)
Thermo Fisher Scientific	QuantStudio® 5	96 well plate (Catalog No. N8010560) Adhesive film (Catalog No. 4311971)

WARNINGS AND PRECUATIONS

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

PANA RealTyper™ HPV Screening 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 HPVS Mix 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. 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 Screening Kit is shipped on icepackag es 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 Screening Kit should be stored immediately upon receipt below -20°C. 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 packagingbelow -20°C for 90 days or until the expiration date, whichever comes first.

KIT CONTENTS

A total of 96 samples can be tested using a kit.

Table 2. Reagents provided in the PANA RealTyper™ HPV Screening Kit

No.	Name of content	Description	Volume	Label & color of cap	Qty
1	HPVS Mix	HPV PNA probes and primers	950 µl	**HPVS Mix**	2 tubes
2	Taq DNA polymerase	Taq DNA polymerase	100 µl	**HPV Taq**	1 tube
3	Positive Control	Positive Control	200 µl	**HPV P.C**	1 tube
4	Negative Control	Negative Control	200 µl	**HPV N.C**	1 tube

PROCEDURES

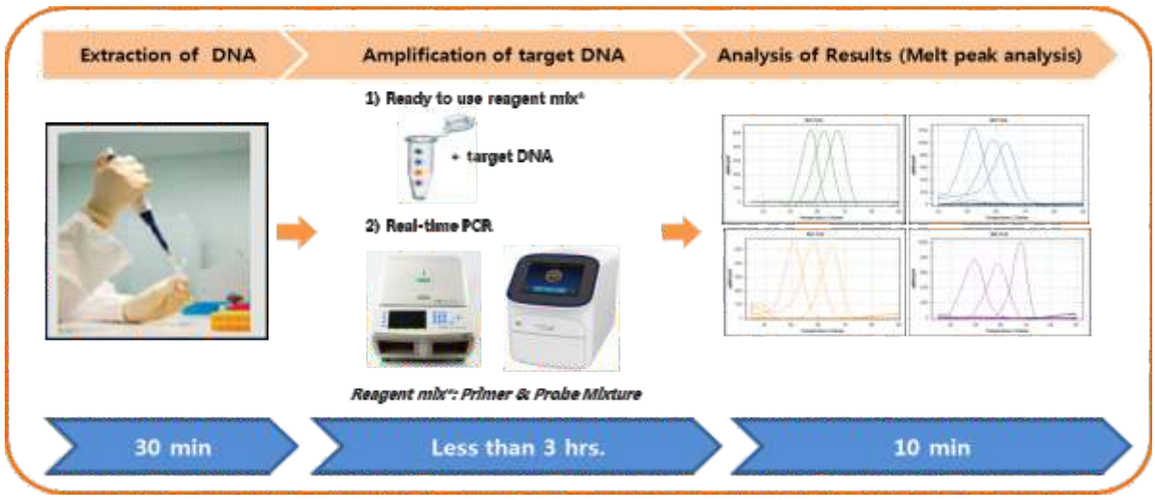


Figure 2. Workflow of the PANA RealTyper™ HPV Screening Kit

1. Sample Preparation and Storage

1) Cervical swab can be used as specimen.
2) Ideally, cervical specimens should be processed on the same day when they are collected.
3) DNA can be isolated using one of recommended kits in Table 3.

Table 3. The list of recommended DNA isolation kit

Model	Company
TANBead® OptiPure Nucleic Acid Kit	Taiwan Advanced Nanotech Inc. (Taiwan)
QIAamp DNA Mini QIAcube Kit	QIAGEN (USA)
MaxWell® 16 Viral Total Nucleic Acid Purification Kit	Promega (USA)

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

2. Preparation of the Real-Time PCR Mixture

Table 4. Set up reaction mixture per one reaction

Reagent	Volume*
HPVS Mix	19 µl
Taq DNA polymerase	1 µl
Extracted DNA, Positive Control(PC), or Negative Control(NC)	5 µl
Total volume	25 µl

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

1) Prepare the reagent mix (HPVS Mix) after thaw, vortex and spin down at room temperature.
2) Prepare test sample (extracted DNA) and control samples (NC and PC).
3) Prepare the PCR plate. Label them as A1 if it is necessary.
4) Load 19 µl of the reagent mix (HPVS Mix) into the PCR plate.
   For example, A1 well will contain HPVS Mix.
5) Add 1 µl of Taq Polymerase to the PCR plate.
6) Add 5 µl of prepared test sample into each well of the PCR plate to yield a total 25µ l of final volume.
7) One set of PC and NC for the HPVS Mix should be included in each run. Add 5 µl of PC or NC into wells of the PCR plate to yield a total 25 µl of final volume.
8) Immediately 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 PCR plate on the block of a real-time PCR instrument.

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

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

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

3) 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 threshold of melting peak analysis according to each dye, respectively (Table 6).

Table 6. The baseline threshold values for Tm determination

CFX96		QuantStudio® 5	
Fluorescent Dye	**Baseline threshold values**	**Fluorescent Dye**	**Baseline threshold values**
FAM	80	FAM	8,000
HEX	50	VIC	8,000
ROX	80	ROX	5,000
Cy5	100	Cy5	10,000

2) Assess the results according to the fluorescent dyes and melting temperatures listed on Tables 7 and 8.

A. Negative Control and Positive Control

The melting temperatures for Negative Control (NC) and Positive Control (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 range s of melting temperature for NC and PC

Type	Dye	Tm (℃)	Type	Dye	Tm (℃)
Negative Control			**Positive Control**		
-	FAM	-	HPV 11	FAM	56.0 - 71.0
-	HEX (or VIC)	-	HPV 18	HEX (or VIC)	44.0 - 54.0
-	ROX	-	HPV other	ROX	41.0 - 73.0
Internal Control	Cy5	52.5 – 67.5	Internal Control	Cy5	52.5 - 67.5

B. Sample

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

Table 8. Criteria of HPV Detection

Type	Dye	Tm (℃)	Type	Dye	Tm (℃)
HPV 6	FAM	42.0 - 55.0	HPV other: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68	ROX	41.0 - 73.0
HPV 11		56.0 - 71.0			
HPV 16	HEX (or VIC)	55.0 - 68.0			
HPV 18		44.0 - 54.0	Internal Control	Cy5	52.5 - 67.5

* 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

Internal Control	HPVS Mix	Interpretation
Valid	Positive	HPV detected*
Valid	Negative	HPV not detected†
Invalid	Positive	HPV detected*
Invalid	Negative	Invalid#

HPV detected (*)

Positive results in HPVS Mix indicate presence of HPV DNA in the clinical sample when the internal control shows valid or invalid results. The HPV type should be detected from criteria in Table 8.

HPV not detected: tested 16 HPV types are not detected (†)

Negative results in HPVS Mix indicate no detection of HPV DNA in the clinical sample when the internal control shows valid results. However, it cannot rule out the possibility that non-targeted HPV types 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 (#)

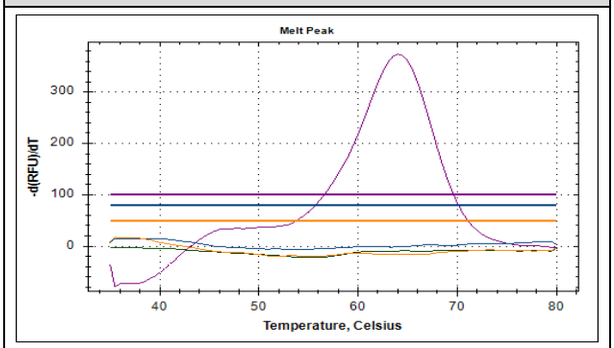
Negative results in HPVS Mix indicate no detection of internal control-targeted DNA in the clinical sample when the internal control show invalid results. It may denote that the amplification of the clinical sample is failed or the amount of isolated DNA is not sufficient. Please repeat the entire test procedure for that clinical sample, starting with DNA isolation.

EXAMPLES OF ANALYSIS

1. CFX96

1) Negative Control (NC)

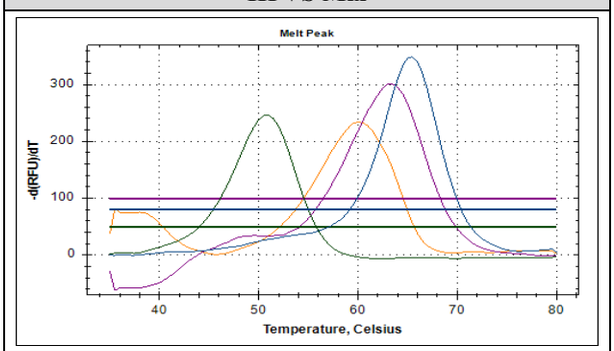
HPVS Mix



HPVS Mix			Result
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**Acceptable**
HEX	-	Negative	
ROX	-	Negative	
Cy5	64.0	I.C Valid	

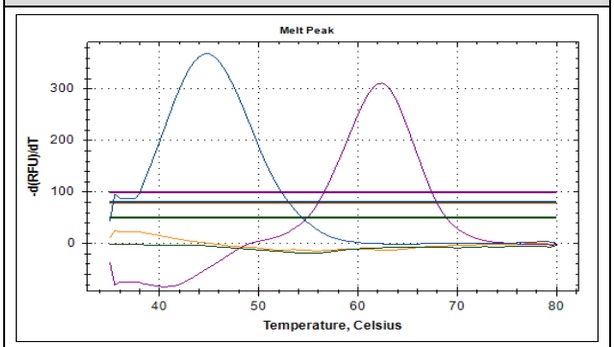
2) Positive Control (PC)

HPVS Mix

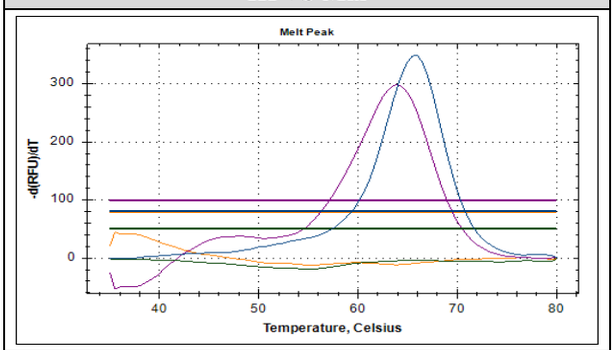


HPVS Mix			Result
Dye	**Tm (°C)**	**Assessment**	
FAM	65.5	Positive	**Acceptable**
HEX	51.0	Positive	
ROX	60.0	Positive	
Cy5	63.5	I.C Valid	

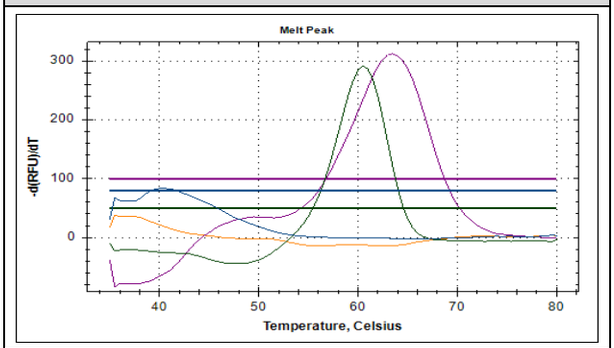
3) Sample 1

HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	44.5	Positive	**HPV 6**
HEX	-	Negative	
ROX	-	Negative	
Cy5	62.5	I.C Valid	

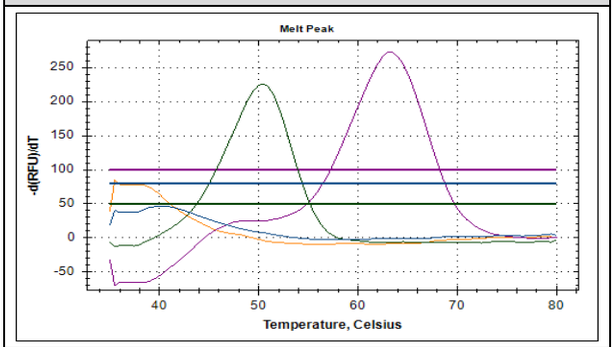
4) Sample 2

HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	66.0	Positive	**HPV 11**
HEX	-	Negative	
ROX	-	Negative	
Cy5	64.0	I.C Valid	

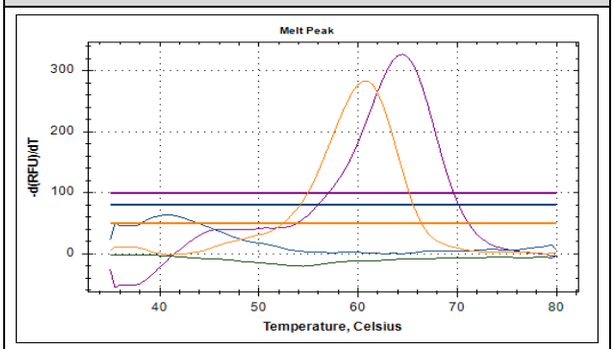
5) Sample 3

HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**HPV 16**
HEX	60.5	Positive	
ROX	-	Negative	
Cy5	63.5	I.C Valid	

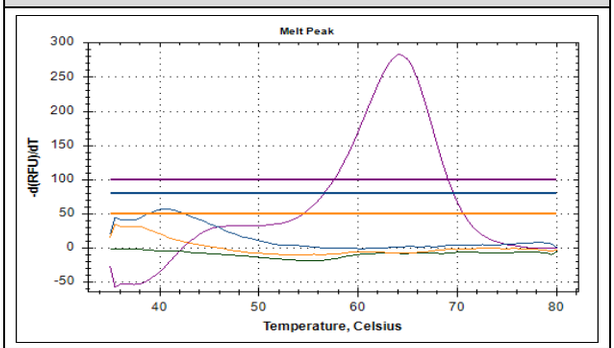
6) Sample 4

HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**HPV 18**
HEX	50.5	Positive	
ROX	-	Negative	
Cy5	63.5	I.C Valid	

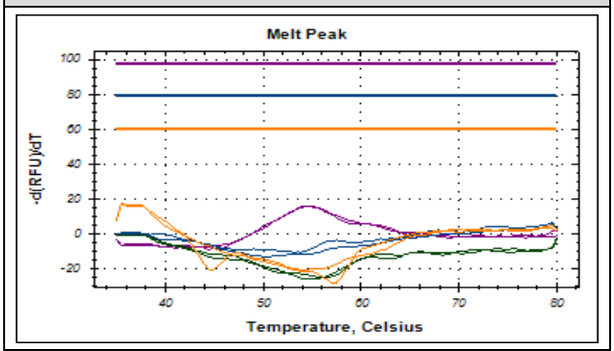
7) Sample 5

HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**HPV other**
HEX	-	Negative	
ROX	61.0	Positive	
Cy5	64.5	I.C Valid	

8) Sample 6

HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**HPV not detected**
HEX	-	Negative	
ROX	-	Negative	
Cy5	64.0	I.C Valid	

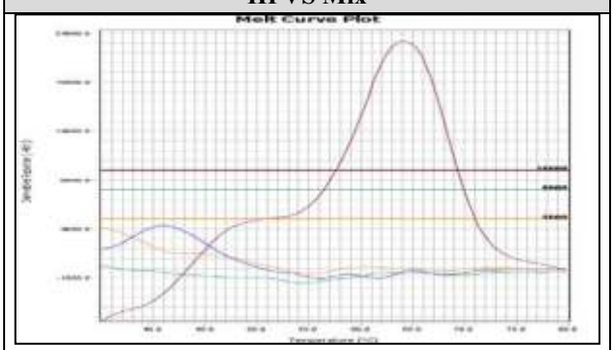
9) Sample 7

HPVS Mix


HPVS Mix			Result
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**Invalid**
HEX	-	Negative	
ROX	-	Negative	
Cy5	-	I.C Invalid	

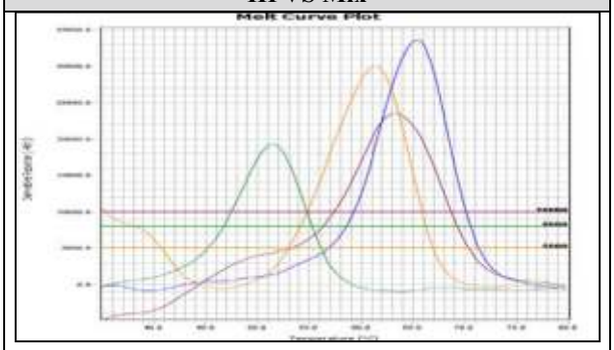
2. QuantStudio® 5

1) Negative Control (NC)

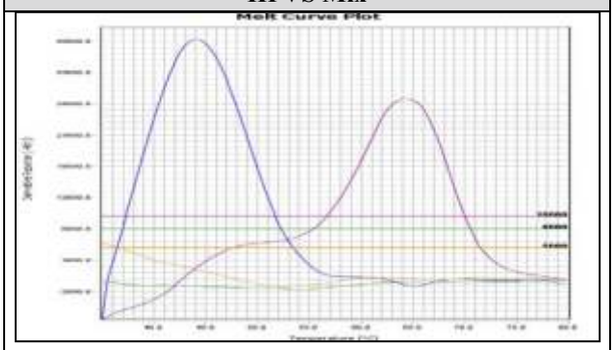
HPVS Mix


HPVS Mix			Result
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**Acceptable**
VIC	-	Negative	
ROX	-	Negative	
Cy5	64.0	I.C Valid	

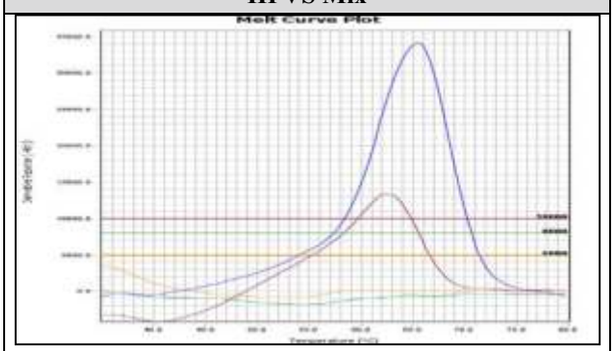
2) Positive Control (PC)

HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	65.3	Positive	**Acceptable**
VIC	51.4	Positive	
ROX	61.3	Positive	
Cy5	63.3	I.C Valid	

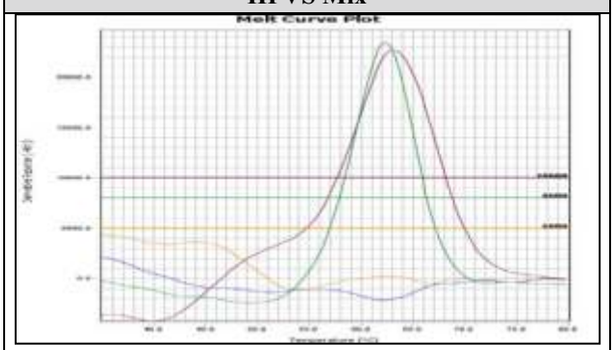
3) Sample 1

HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	44.2	Positive	**HPV 6**
VIC	-	Negative	
ROX	-	Negative	
Cy5	64.3	I.C Valid	

4) Sample 2

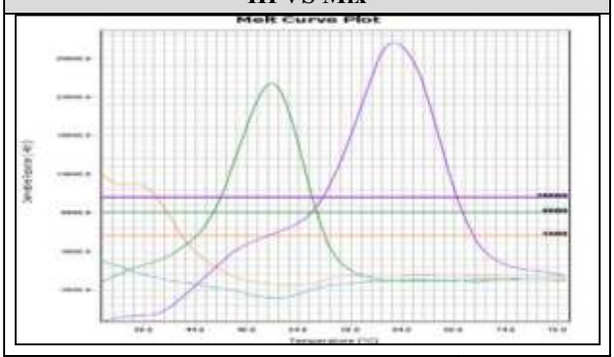
HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	65.5	Positive	**HPV 11**
VIC	-	Negative	
ROX	-	Negative	
Cy5	62.5	I.C Valid	

5) Sample 3

HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**HPV 16**
VIC	62.3	Positive	
ROX	-	Negative	
Cy5	63.0	I.C Valid	

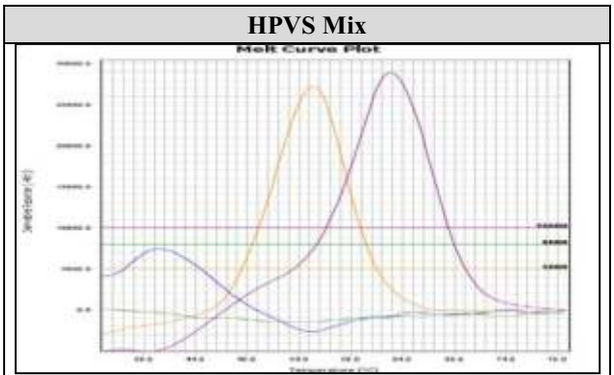
6) Sample 4

HPVS Mix			Result
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**HPV 18**
VIC	51.4	Positive	
ROX	-	Negative	
Cy5	63.3	I.C Valid	

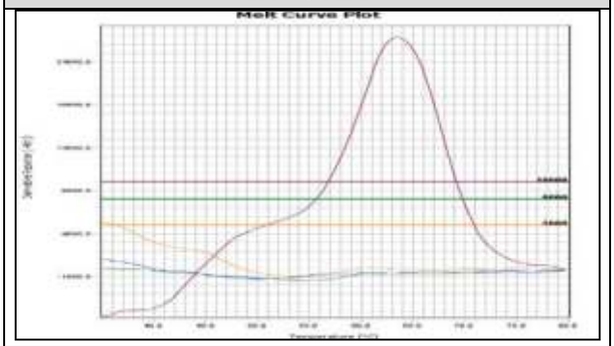


7) Sample 5

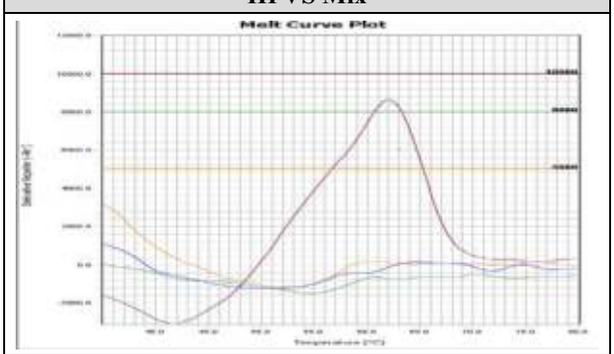
HPVS Mix			Result
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**HPV other**
VIC	-	Negative	
ROX	55.3	Positive	
Cy5	62.8	I.C Valid	



8) Sample 6

HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**HPV not detected**
VIC	-	Negative	
ROX	-	Negative	
Cy5	63.5	I.C Valid	

9) Sample 7

HPVS Mix			
			
HPVS Mix			**Result**
Dye	**Tm (°C)**	**Assessment**	
FAM	-	Negative	**Invalid**
VIC	-	Negative	
ROX	-	Negative	
Cy5	-	I.C Invalid	

QUALITY CONTROL

Each lot of PANA RealTyper™ HPV Screening Kit 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 limit of detection study was evaluated using three batches of kits and the standard materials, plasmids and cell linesc ontaining the target gene. The detection limitfor each target gene is summarized in Table 10.

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

Type	Limit of detection (LOD)	Type	Limit of detection (LOD)
HPV 6	5×10^1 copies/rxn	HPV 52	5×10^1 copies/rxn
HPV 11	5×10^1 copies/rxn	HPV 56	5×10^1 copies/rxn
HPV 16	5×10^1 copies/rxn	HPV 58	5×10^2 copies/rxn
HPV 18	5×10^1 copies/rxn	HPV 59	5×10^2 copies/rxn
HPV 31	5×10^1 copies/rxn	HPV 66	5×10^1 copies/rxn
HPV 33	5×10^2 copies/rxn	HPV 68	5×10^2 copies/rxn
HPV 35	5×10^2 copies/rxn	HPV 16 (SiHa)	0.01 ng/rxn
HPV 39	5×10^2 copies/rxn	HPV 18 (HeLa)	0.005 ng/rxn
HPV 45	5×10^1 copies/rxn	HPV 68 (ME-180)	0.05 ng/rxn
HPV 51	5×10^2 copies/rxn		

2. Analytical Specificity

The cross reactivity study was evaluated using three batches of kits and DNA samples isolated from 49 different microorganisms. The results showed no cross reactivity with any below tested pathogenic microorganisms.

Bacteria Organism	Virus Organism
Lactobacillus acidophilus	Adenovirus, type 5
Staphylococcus epidermidis	Cytomegalovirus
Staphylococcus aureus	Human herpesvirus 1
Streptococcus faecalis	Human herpesvirus 2
Streptococcus pyogenes	HPV 26
Streptococcus agalactiae	HPV 30
Corynebacterium glutamicum	HPV 32
Neisseria gonorrhoeae	HPV 34
Escherichia coli	HPV 40
Enterococcus faecium	HPV 42
Clostridium perfringens	HPV 43
Peptostreptococcus prevotii (Anaerococcus prevotii)	HPV 44
Klebsiella pneumoniae	HPV 53
Enterobacter cloacae	HPV 54
Proteus mirabilis	HPV 55
Pseudomonas aeruginosa	HPV 61
Bacteroides fragilis	HPV 62
Bifidobacterium adolescentis	HPV 67
Fusobacterium varium	HPV 69
Treponema pallidum	HPV 70
Candida albicans	HPV 73
	HPV 74
	HPV 81
	HPV 82
	HPV 83
	HPV 84
	HPV 87
	HPV 90

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EXPLANATION OF SYMBOLES ON THE LABEL

IVD	*In Vitro* Diagnostic Medical Device		Manufacturer
LOT	Batch code	Σ	Contains Sufficient for <*n*> tests
REF	Catalogue number		Upper limit of storage temperature
EC REP	Authorized European representative		Use by
	Consult instructions for use	CE	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.

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