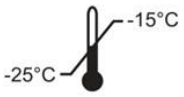
Instructions For Use

CE IVD *For In Vitro Diagnostic Use*



Store at -25°C to -15°C

REF HPVG29-RT50

Σ 50

geneMAP™ HPV 29 Genotyping Kit

For Real-Time PCR

Multiplex Real-time PCR System for detection and genotyping of 29 High and Low Risk Human Papilloma Virus (HPV16, HPV18, HPV26, HPV31, HPV33, HPV35, HPV39, HPV40, HPV42, HPV43, HPV44, HPV45, HPV51, HPV52, HPV53, HPV54, HPV56, HPV58, HPV59, HPV61, HPV66, HPV68, HPV69, HPV70, HPV73, HPV81/82 and HPV6/11) from urine, fresh tissue, FFPE tissue, buccal swab, cervical swab and liquid based cytology specimens.

Validated on 4 channel Realtime PCR instruments such as:

* Biorad® CFX96, Real-time PCR System (Bio-Rad)
* Biomolecular Systems/Mic PCR
* ThermoScientific ABI7500 and QuantStudio Series
* RotorGene Q5/Q6 Realtime PCR System (Qiagen)- 72-Well Rotor
* Roche, LightCycler® 480 II, Cobas Z480

Contents

1. Intended Use

The HPV 29 Genotyping Kit is a semi qualitative in vitro assay for detection and genotyping of 29 High Risk and Low Risk Human Papilloma Viruses (HPV16, HPV18, HPV26, HPV31, HPV33, HPV35, HPV39, HPV40, HPV42, HPV43, HPV44, HPV45, HPV51, HPV52, HPV53, HPV54, HPV56, HPV58, HPV59, HPV61, HPV66, HPV68, HPV69, HPV70, HPV73, HPV81/82 and HPV6/11) from urine, fresh tissue, FFPE tissue, buccal swab, cervical swab and liquid based cytology specimens.

2. Safety Instructions & General Warnings

- This kit must be used strictly in accordance with the instructions provided in this manual, and only in combination with validated reagents and instruments. Any off-label use of this product, as well as any modification of its components, will nullify Genmark's liability.
- Obey proper laboratory safety protocols when working with chemicals and specimens.
- The protocol can be performed by only professional and trained personal.
- Perform the protocol in a well-ventilated and well-lit environment.
- Store the kit and its components within recommended temperature range in de-frost refrigerators. Do not use no-frost refrigerators.
- Avoid skin contact with any of the reagents in the kit
- Wipe workspace surfaces with 10% bleach followed by 70% alcohol.
- Instruments may exhibit performance variations due to differences in electrical currents and power outlets, as well as the effects of maintenance and calibration. It is the responsibility of the user to ensure that all instruments are properly maintained and regularly calibrated according to the manufacturer's guidelines. Genmark disclaims responsibility for any performance issues arising from improper maintenance, calibration, or variations in electrical supply.
- All biological specimens should be handled as potentially infectious, following standard precautions. For guidelines on specimen handling, refer to the World Health Organization.
- Consult environmental waste personnel for proper disposal of used plates, consumables, and reagents, considering federal and local hazardous waste regulations. Check local and national disposal requirements.

3. Principles and Technology

3.1 Principles

The polymerase chain reaction (PCR) is the sensitive and specific TaqMan Probe technology with the use of DNA amplification technique, primer design and PCR optimization, and innovations and sensors related to PCR. The HPV 29 Genotyping kit, based on two main processes: nucleic acid extraction and PCR amplification of nucleic acid in the primer and probe mechanism of PCR machines by real PCR.

Samples

(Genital/buccal/cervical swab, urine and liquid based cytology specimens)

↓ Nucleic acid extraction

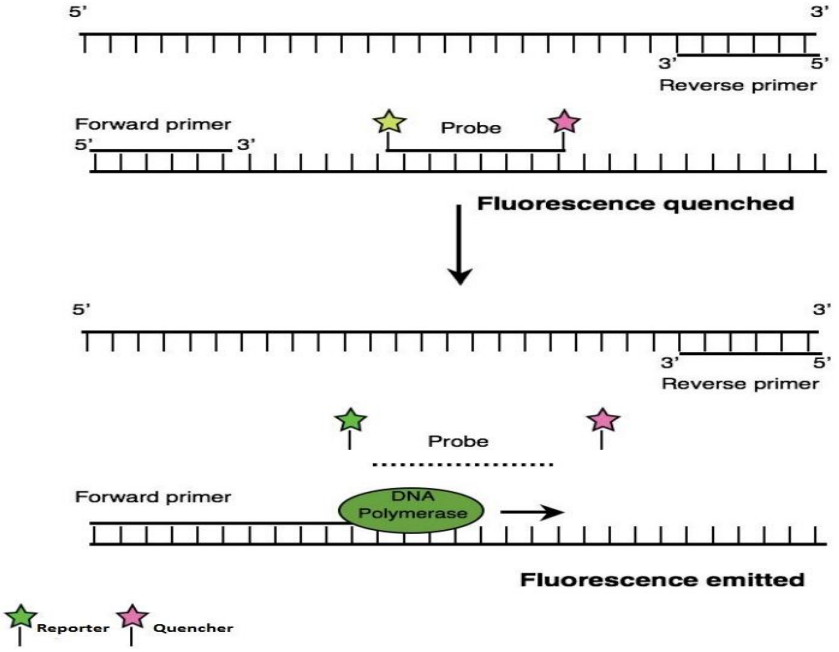
Nucleic acid

↓ Amplification and detection using TaqMan Probe system

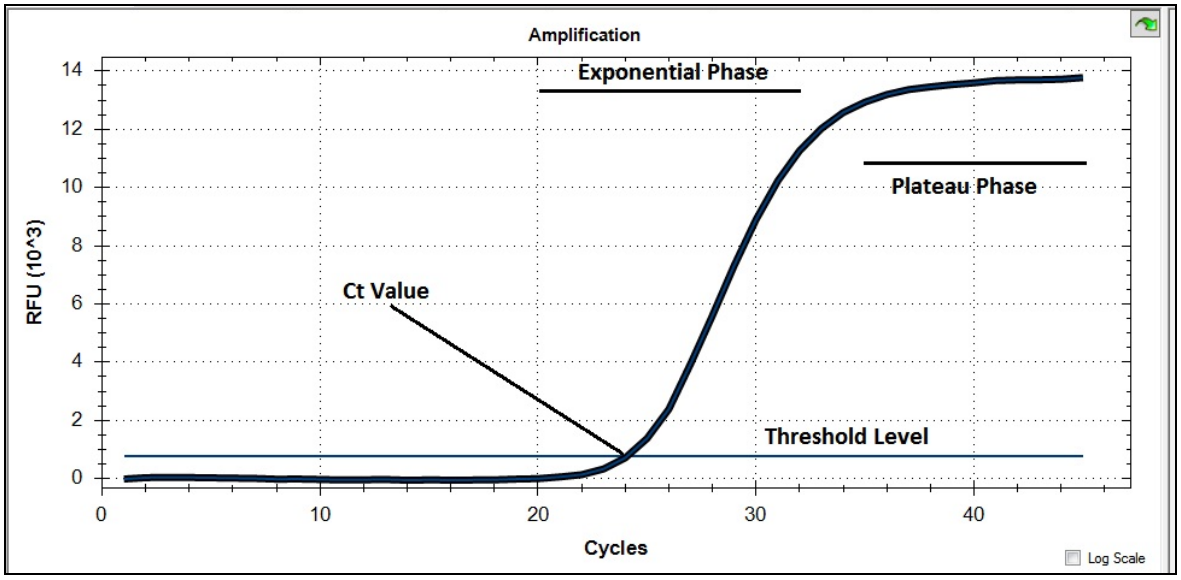
Analysis of results

3.2 Technology

Hydrolysis (TaqMan) probes are the most common form of qPCR probes and are widely used in human, veterinary, and environmental diagnostics. These probes utilize a fluorescent dye at one end of the DNA oligonucleotide and a quencher at the other. During PCR, the probe specifically anneals to the target DNA sequence (from sample), which is flanked by the two primers. As DNA polymerase extends the new DNA strand, the probe is degraded by the 5' to 3' exonuclease activity of the polymerase, resulting in the fluorophore being separated from the quencher and emitting fluorescence. The more DNA present in the reaction, the earlier the fluorescence reaches a detectable level resulting in earlier Ct values.



Hydrolysis (TaqMan) Probe Technology.



Typical Amplification Plot of Real-Time PCR in Linear Scale Graphic.

4. Background Information

Worldwide, cervical cancer is the fourth most frequent cancer in women with an estimated 604 000 new cases in 2020. Of the estimated 342,000 deaths from cervical cancer in 2020, about 90% of these occur in low- and middle-income countries. Women living with HIV are 6 times more likely to develop cervical cancer compared to women without HIV, and an estimated 5% of all cervical cancer cases are attributable to HIV (2). Moreover, in all world regions the contribution of HIV to cervical cancer falls disproportionately on younger women.

In high-income countries, programmes are in place which enable girls to be vaccinated against HPV and women to get screened regularly and treated adequately. Screening allows pre-cancerous lesions to be identified at stages when they can easily be treated.

In low-and middle-income countries, there is limited access to these preventative measures and cervical cancer is often not identified until it has further advanced, and symptoms develop. In addition, access to treatment of cancerous lesions (for example, cancer surgery, radiotherapy, and chemotherapy) may be limited, resulting in a higher rate of death from cervical cancer in these countries.

The high mortality rate from cervical cancer globally (age standardized rate among women: 13.3/100 000 in 2020) could be reduced by effective interventions at different stages of life.

A large majority of cervical cancer (more than 95%) is due to the human papillomavirus (HPV).

HPV is the most common viral infection of the reproductive tract. Most sexually active women and men will be infected at some point in their lives, and some may be repeatedly infected. More than 90% of the infected populations eventually clear the infection.

Cervical cancer is by far the most common HPV-related disease. Nearly all cases of cervical cancer can be attributed to HPV infection.

Although most HPV infections clear up on their own and most pre-cancerous lesions resolve spontaneously, there is a risk for all women that HPV infection may become chronic and pre-cancerous lesions progress to invasive cervical cancer.

It takes 15 to 20 years for cervical cancer to develop in women with normal immune systems. It can take only 5 to 10 years in women with weakened immune systems, such as those with untreated HIV infection. (1)

HPV is a group of more than 200 related viruses, some of which are spread through vaginal, anal, or oral sex. Sexually transmitted HPV types fall into two groups, low risk and high risk.

Low-risk HPVs mostly cause no disease. However, a few low-risk HPV types can cause warts on or around the genitals, anus, mouth, or throat.

High-risk HPVs can cause several types of cancer. There are about 14 high-risk HPV types including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Two of these, HPV16 and HPV18, are responsible for most HPV-related cancers. (2)

The kit targets following HPV Types;

Pathogen	Targeted Gene
HPV 6&11	Major Capsid Protein L1 Gene
HPV 16	Major Capsid Protein L1 Gene
HPV 18	Major Capsid Protein L1 Gene
HPV 26	Major Capsid Protein L1 Gene
HPV 31	Major Capsid Protein L1 Gene
HPV 33	Major Capsid Protein L1 Gene
HPV 35	Major Capsid Protein L1 Gene
HPV 39	Major Capsid Protein L1 Gene
HPV 40	Major Capsid Protein L1 Gene
HPV 42	Major Capsid Protein L1 Gene
HPV 43	Major Capsid Protein L1 Gene
HPV 44	Major Capsid Protein L1 Gene
HPV 45	Major Capsid Protein L1 Gene
HPV 51	Major Capsid Protein L1 Gene
HPV 52	Major Capsid Protein L1 Gene
HPV 53	Major Capsid Protein L1 Gene
HPV 54	Major Capsid Protein L1 Gene
HPV 56	Major Capsid Protein L1 Gene
HPV 58	Major Capsid Protein L1 Gene
HPV 59	Major Capsid Protein L1 Gene
HPV 61	Major Capsid Protein L1 Gene
HPV 66	Major Capsid Protein L1 Gene
HPV 68	Major Capsid Protein L1 Gene
HPV 69	Major Capsid Protein L1 Gene
HPV 70	Major Capsid Protein L1 Gene
HPV 73	Major Capsid Protein L1 Gene
HPV 81 & 82	Major Capsid Protein L1 Gene

5. Reagents

Reagents contained in a kit are sufficient for 50 reactions

Description	No. of Reactions	No. of Tubes	Vol. Per Tube	Color of Caps	Description
4X HPV Primer Probe Mix 1	50	1	275 µl	Amber	Primer Probe Mix (PPM): • Amplification and detection reagents • Template of Internal Control
4X HPV Primer Probe Mix 2	50	1	275 µl	Amber	Primer Probe Mix (PPM): • Amplification and detection primer probe sets
4X HPV Primer Probe Mix 3	50	1	275 µl	Amber	Primer Probe Mix (PPM): • Amplification and detection primer probe sets
4X HPV Primer Probe Mix 4	50	1	275 µl	Amber	Primer Probe Mix (PPM): • Amplification and detection primer probe sets
4X HPV Primer Probe Mix 5	50	1	275 µl	Amber	Primer Probe Mix (PPM): • Amplification and detection primer probe sets
4X HPV Primer Probe Mix 6	50	1	275 µl	Amber	Primer Probe Mix (PPM): • Amplification and detection primer probe sets
4X HPV Primer Probe Mix 7	50	1	275 µl	Amber	Primer Probe Mix (PPM): • Amplification and detection primer probe sets
2X Master Mix with UDG	45	3	1100 µl	Clear	• DNA polymerase, UDG • Buffer containing dNTPs
2X Master Mix with UDG	5	1	550 µl	Clear	• DNA polymerase, UDG • Buffer containing dNTPs
RNase Free Water	20	1	400 µl	Violet	• RNase Free Water for Negative Template Control
HPV Positive Control	10	1	220 µl	Red	Positive Control (PC): • Mixture of pathogen and IC clones
DNA Extraction Solution (DES-120)	60	1	7 mL	White	• Reagent for bacterial DNA extraction

6. Storage and Handling

All components must be stored at between -15° C /-25 ° C. All components are stable under the recommended storage conditions until the expiration date indicated on the label on the box. The performance of the kit components is not affected until 5 freeze and thaw. If reagents are to be used only intermittently, they should be stored in aliquots.

7. Materials Required but Not Provided

- 1X PBS solution
- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and sterile pipette tips
- 1.5mL microcentrifuge tubes
- Heat block
- Desktop centrifuge
- Vortex mixer
- Clean bench
- Real Time PCR plastics which is manufacturer recommended
  For Biorad CFX Instruments;
- 96-Well Skirted PCR Plate, White well (Cat. No. HSP-9655, Biorad)
- Permanent Clear Heat Seal (Cat. No. 1814035, Biorad) or Microseal 'B' Film (MSB1001, Biorad)
- Low-Profile PCR Tubes 8-tube strip, white (BPB59009-1, Bioplastics)
- Optical Flat 8-Cap Strips for 0,2ml tube strips/plates (BPB57651, Bioplastics) (TCS0803, Biorad)

8. Protocol

8.1 Specimen Collection, Storage, and Transport

All samples should be considered as potentially infectious material. Only sample materials collected, stored and transported in accordance with the following rules and instructions are permitted.

To ensure a high sample quality, samples should be transported as quickly as possible. The samples should be transported at the specified temperatures.

8.1.1 Specimen Storage and Transport

<table>
<tr><th>Specimen</th><th colspan="2">Storage and Transport Temp
Duration</th><th>Note</th></tr>
<tr><td>SurePath</td><td rowspan="5">2-8 °C</td><td>3 weeks</td><td rowspan="2">-Performance may be affected by prolonged storage of specimens.</td></tr>
<tr><td>ThinPrep</td><td>6 weeks</td></tr>
<tr><td>Genital Swab</td><td rowspan="3">2 weeks</td><td rowspan="3">-Specimens should also adhere to local and national instructions for transport of pathogenic meterial.</td></tr>
<tr><td>Fresh Tissue/Cervical/Buccal Swab</td></tr>
<tr><td>Urine specimen</td></tr>
<tr><td>FFPE Tissue</td><td>Room temperature</td><td>One year</td><td></td></tr>
</table>

*** Duration:** The time interval, including sample storage and transport, prior to sampling.

8.2 Pretreatment of Specimens

8.2.1 Liquid based cervical cytology specimen

- Equilibrate samples to room temperature (19~25℃).
- Centrifuge 1 mL of liquid based cervical cytology specimen for 10 minutes at 13,000 rpm in 1,5mL Microcentrifuge tube.
- The supernatant must be discarded. Afterwards, the pellet must be resuspended in 500uL dd$H_2$0 or PBS by vortexing thoroughly.
- Centrifuge at 13,000 rpm for 5 minutes.
- Discard supernatant and get the pellet for DNA extraction step.
- Follow the manufacturer's protocol.

8.2.2 Genital swab specimens

- Genital swab specimen is used without pre-treatment.

8.2.3 FFPE Tissue

- Cut up to 1-3 sections of 5-10 µm in thickness from the FFPE sample block.
- Immediately place the sections in a 1.5 ml or 2 ml microcentrifuge tube.
- Add 1000 µl Xylene to the sample, close the lid and vortex vigorously for 10 seconds.
- Centrifuge at full speed (>13,000 x g) for 2 minutes at room temperature.
- Discard the supernatant by pipetting. Do not remove any of the pellet(tissue).
- Repeat step 3- 5.
- Add 1000 µl ethanol (96–100%) to the pellet, and mix by vortexing.
- The ethanol extracts residual xylene from the sample.
- Centrifuge at full speed (>13,000 x g) for 2 minutes at room temperature.
- Remove the supernatant by pipetting. Do not remove any of the pellet.
- Incubate the pellet at room temperature with the tube cap open until residual ethanol is completely removed
- Follow the manufacturer's protocol.

8.2.4 Urine specimens

- Equilibrate samples to room temperature (19~25℃).
- Centrifuge 1 mL of urine specimen for 5 minutes at 13,000 rpm in 1,5mL Microcentrufuge tube.
- The supernatant must be discarded. Afterwards, the pellet must be resuspended in 1000uL ddH20 or PBS by vortexing thoroughly.
- Centrifuge at 13,000 rpm for 5 mimutes.
- Discard supernatant and get the pellet/sediment for DNA extraction step.
- Follow the manufacturer's protocol.

8.3 Nucleic Acid Extraction

8.3.1 Manual Nucleic Acid Extraction Kits (Genmark Extraction Solution)

The DNA Extraction Solution is available in the kit as free of charge.

- Add 100 µL of DNA Extraction Solution (freshly vortexed) into the specimen pellet/sediment/tissue and vortex for 30 seconds.
- Lock the tube cap using a cap-lock and boil at 100°C for 15 minutes on heat block.
- Centrifuge at 13,000 rpm for 5 minutes.
- Use 5 µL of supernatant as PCR template for each reaction.

8.3.2 Commercial DNA Extraction Kits

Column and magnetic bead based commercial extraction kits (Manual or Automated) can be used for DNA extraction step. According to extraction kit protocol, specimen pellet/sediment/tissue can be mix with PBS to reach starting material volume.

8.4 Preparation for Real-time PCR

-The correct tubes and caps must be used (see MATERIALS REQUIRED BUT NOT PROVIDED).
-Aerosol resistant filter tips and tight gloves must be used when preparing 2X Master Mix with UDG reactions.
-Use extreme care to ensure no cross-contamination.
-Completely thaw all reagents at room temperature.
-Briefly centrifuge the reagent tubes to remove drops from the inside of the cap.
-Prepare 1.5mL Microcentrifuge tubes for Samples, PC and NTC
-Thaw the primer/probe mix tubes, positive control mixes, and 2X Master Mix with UDG.
-Vortex and spin down the tubes for about 5 seconds at room temperature.
-Five reactions are set up for each sample.
-The PCR reactions are setup in a total volume of 20 µl/sample.
-Reaction mixes for multiple samples (as well as control samples) should be pre-mixed as a master mix with 5% excess volume to compensate for pipetting losses.
-Alternatively, individual master mixes can be made with each primer/probe mix, with DNA samples added directly to the plate.
-The following reagents go into each 20 µl reaction:

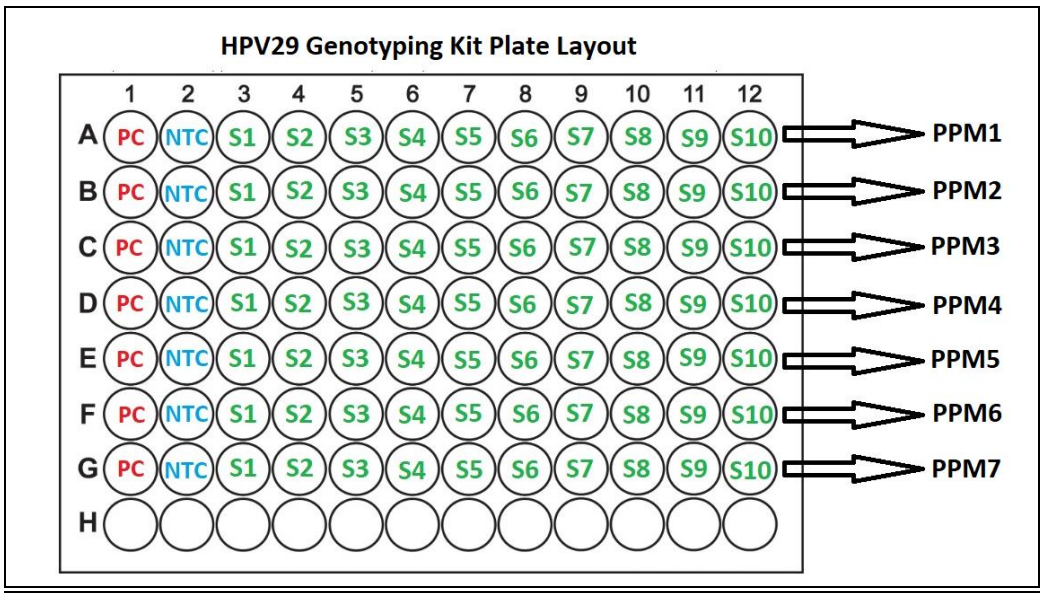
Component	Volume
2X Master Mix with UDG	10 µL
Primer Probe Mix (1-7)	5 µL
Sample Nucleic Acid	5 µL
Total Volume	20 µL

-Prepare a master mix for each sample as follows (calculated with 5% overage):

Reagent	Volume per Sample
2X Master Mix with UDG	73.5 µL
Sample Nucleic Acid or PC or NTC	36.75 µL

-Pulse vortex to mix and perform a quick spin down, at room temperature.
- Dispense 15 µl of the master mix per well into a single column (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and12) as shown in the Table 1.
- Add 5 µl of each primer/probe mix to its corresponding row wells (A, B, C, D, E, F & G)

Table 1

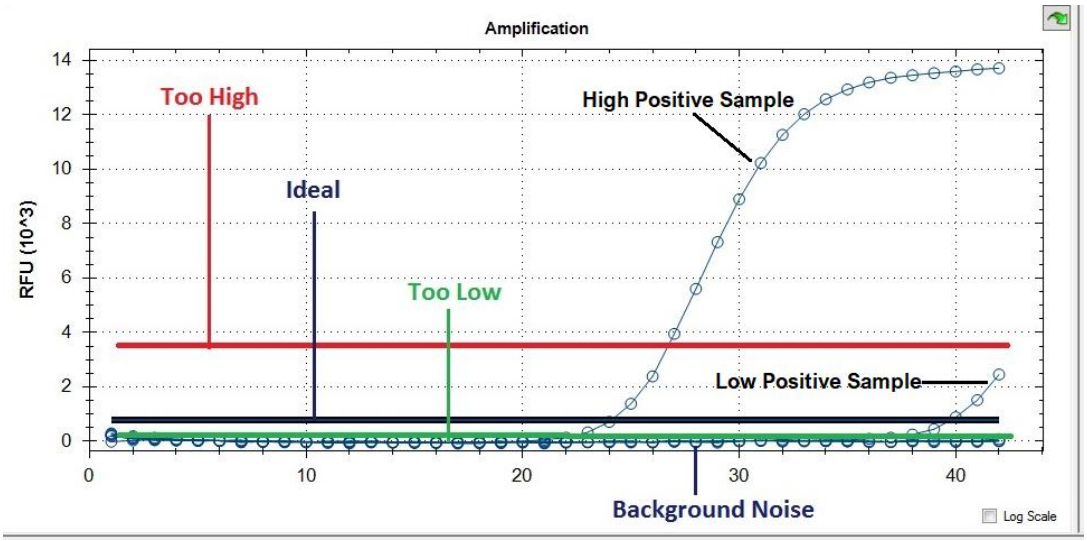


8.5 General Rules of the Threshold Settings Manually

Normally the software-based methods will select a proper threshold, but in cases where the curves do not conform to the assumptions made by the algorithm, an incorrect threshold may be calculated. Good indicators of improperly-set threshold values are false positives (Ct values obtained from negative control wells), known positive samples giving very late Cts or no Cts at all. Because of those reasons manually adjusting the threshold is highly recommended.

Ideally, the threshold should be set in the region where the just above the background noise. The threshold should not be so high that it crosses any of the plots where they are starting to plateau and are no longer linear.

To adjust the threshold for each dye collected must be set separately.



Example of ideal threshold level.

If threshold is too high, it gives false negative (Missing the low positive sample).
If threshold is too low, it gives false positive (Intercept the background noise).

8.6 Real-Time PCR Instrument Setup and Results Analysis

1. In Protocol Editor, define the thermal profile as follows:

1) *For Biorad CFX96, QuantStudio Series and Other Four Channels Insturments*

Temperature	Time	Cycles	Data Collection
25˚C	5 min	1	
95˚C	4 min	1	
95˚C	5 sec	50	
56˚C	20 sec		FAM, VIC, ROX, CY5

2) *For ABI7500 (Standard Mode)*

Temperature	Time	Cycles	Data Collection
25˚C	5 min	1	
95˚C	4 min	1	
95˚C	5 sec	50	
56˚C	32 sec		FAM, VIC, ROX, CY5

Note1*: Passive reference dye should be set as "none"*

3) *For Rotorgene Q5/Q6 (Duration 75 min.)*

Temperature	Time	Cycles	Data Collection
50˚C	5 min	1	
95˚C	4 min	1	
95˚C	5 sec	50	
56˚C	20 sec		Green, Yellow, Orange, Red

Note1*: Please use only 72-well carousel, 36-well carousel is not recommended*

Note2: *Please perform Auto-Gain optimization before first acquisition. (Auto-Gain optimization tube should be PC)*

4) *For Lightcycler480 (Roche)*

Temperature	Time	Cycles	Data Collection
25˚C	5 min	1	
95˚C	4 min	1	
95˚C	5 sec	50	
56˚C	20 sec		FAM (465-510), VIC/HEX (533-580), ROX (533-610), CY5 (618-660)

Note1: *4 channels Color Compansation must be performed before the study*

Plate Read at Step 3. Fluorescence is detected at 56°C.

Interpretation Table

	FAM	VIC/HEX	ROX	CY5
Mix1	HPV 45	HPV 16	HPV 18	Endogenous Control (Human B2M Gene)
Mix2	HPV 31	HPV 35	HPV 33	HPV 39
Mix3	HPV 59	HPV 66	HPV 56	HPV 58
Mix4	HPV 51	HPV 52	HPV 68	HPV 6 & 11
Mix5	HPV 40	HPV 42	HPV 61	HPV 26
Mix6	HPV 44	HPV 53	HPV 43	HPV 69
Mix7	HPV 81 & 82	HPV 54	HPV 73	HPV 70

Mix 1

Analyte	Fluorophore	Sample Ct Value	Result
HPV 45	FAM	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 16	VIC	<45	Positive (+)
		≥45	Negative (-)
HPV 18	ROX	<45	Positive (+)
		≥45 or NA	Negative (-)
Endogenous Control	CY5	<36	Positive (+)
		≥36 or NA	Negative (-)

Mix 2

Analyte	Fluorophore	Sample Ct Value	Result
HPV 31	FAM	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 35	VIC	<45	Positive (+)
		≥45	Negative (-)
HPV 33	ROX	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 39	CY5	<45	Positive (+)
		≥45 or NA	Negative (-)

Mix 3

Analyte	Fluorophore	Sample	
		Ct Value	Result
HPV 59	FAM	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 66	VIC	<45	Positive (+)
		≥45	Negative (-)
HPV 56	ROX	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 58	CY5	<45	Positive (+)
		≥45 or NA	Negative (-)

Mix 4

Analyte	Fluorophore	Sample	
		Ct Value	Result
HPV 51	FAM	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 52	VIC	<45	Positive (+)
		≥45	Negative (-)
HPV 68	ROX	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 6/11	CY5	<45	Positive (+)
		≥45 or NA	Negative (-)

Mix 5

Analyte	Fluorophore	Sample	
		Ct Value	Result
HPV 40	FAM	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 42	VIC	<45	Positive (+)
		≥45	Negative (-)
HPV 61	ROX	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 26	CY5	<45	Positive (+)
		≥45 or NA	Negative (-)

Mix 6

Analyte	Fluorophore	Sample	
		Ct Value	Result
HPV 44	FAM	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 53	VIC	<45	Positive (+)
		≥45	Negative (-)
HPV 43	ROX	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 69	CY5	<45	Positive (+)
		≥45 or NA	Negative (-)

Mix 7

Analyte	Fluorophore	Sample	
		Ct Value	Result
HPV 81/82	FAM	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 54	VIC	<45	Positive (+)
		≥45	Negative (-)
HPV 73	ROX	<45	Positive (+)
		≥45 or NA	Negative (-)
HPV 70	CY5	<45	Positive (+)
		≥45 or NA	Negative (-)

	FAM	VIC	ROX	CY5	Interpretation
Mix 1	HPV 45 (-)	HPV 16 (-)	HPV 18 (-)	Endogenous Control (+)	HPV negative
Mix 2	HPV 31 (-)	HPV 35 (-)	HPV 33 (-)	HPV 39 (-)	
Mix 3	HPV 59 (-)	HPV 66 (-)	HPV 56 (-)	HPV 58 (-)	
Mix 4	HPV 51 (-)	HPV 52 (-)	HPV 68 (-)	HPV6/11 (-)	
Mix 5	HPV 40 (-)	HPV 42 (-)	HPV 61 (-)	HPV 26 (-)	
Mix 6	HPV 44 (-)	HPV 53 (-)	HPV 43 (-)	HPV 69 (-)	
Mix 7	HPV81/82 (-)	HPV 54 (-)	HPV 73 (-)	HPV 70 (-)	

	FAM	VIC	ROX	CY5	Interpretation
Mix 1	HPV 45 (-)	HPV 16 (+)	HPV 18 (-)	Endogenous Control (+)	HPV 16 Positive
Mix 2	HPV 31 (-)	HPV 35 (-)	HPV 33 (-)	HPV 39 (-)	
Mix 3	HPV 59 (-)	HPV 66 (-)	HPV 56 (-)	HPV 58 (-)	
Mix 4	HPV 51 (-)	HPV 52 (-)	HPV 68 (-)	HPV6/11 (-)	
Mix 5	HPV 40 (-)	HPV 42 (-)	HPV 61 (-)	HPV 26 (-)	
Mix 6	HPV 44 (-)	HPV 53 (-)	HPV 43 (-)	HPV 69 (-)	
Mix 7	HPV81/82 (-)	HPV 54 (-)	HPV 73 (-)	HPV 70 (-)	

	FAM	VIC	ROX	CY5	Interpretation
Mix 1	**HPV 45 (+)**	HPV 16 (-)	HPV 18 (-)	**Endogenous Control (+)**	HPV Co-Infection (HPV 45, 33 and 6/11 positive)
Mix 2	HPV 31 (-)	HPV 35 (-)	**HPV 33 (+)**	HPV 39 (-)	
Mix 3	HPV 59 (-)	HPV 66 (-)	HPV 56 (-)	HPV 58 (-)	
Mix 4	HPV 51 (-)	HPV 52 (-)	HPV 68 (-)	**HPV6/11 (+)**	
Mix 5	HPV 40 (-)	HPV 42 (-)	HPV 61 (-)	HPV 26 (-)	
Mix 6	HPV 44 (-)	HPV 53 (-)	HPV 43 (-)	HPV 69 (-)	
Mix 7	HPV81/82 (-)	HPV 54 (-)	HPV 73 (-)	HPV 70 (-)	

	FAM	VIC	ROX	CY5	Interpretation
Mix 1	HPV 45 (-)	HPV 16 (-)	**HPV 18 (+)**	Endogenous* Control (-)	HPV 18 Positive
Mix 2	HPV 31 (-)	HPV 35 (-)	HPV 33 (-)	HPV 39 (-)	
Mix 3	HPV 59 (-)	HPV 66 (-)	HPV 56 (-)	HPV 58 (-)	
Mix 4	HPV 51 (-)	HPV 52 (-)	HPV 68 (-)	HPV6/11 (-)	
Mix 5	HPV 40 (-)	HPV 42 (-)	HPV 61 (-)	HPV 26 (-)	
Mix 6	HPV 44 (-)	HPV 53 (-)	HPV 43 (-)	HPV 69 (-)	
Mix 7	HPV81/82 (-)	HPV 54 (-)	HPV 73 (-)	HPV 70 (-)	

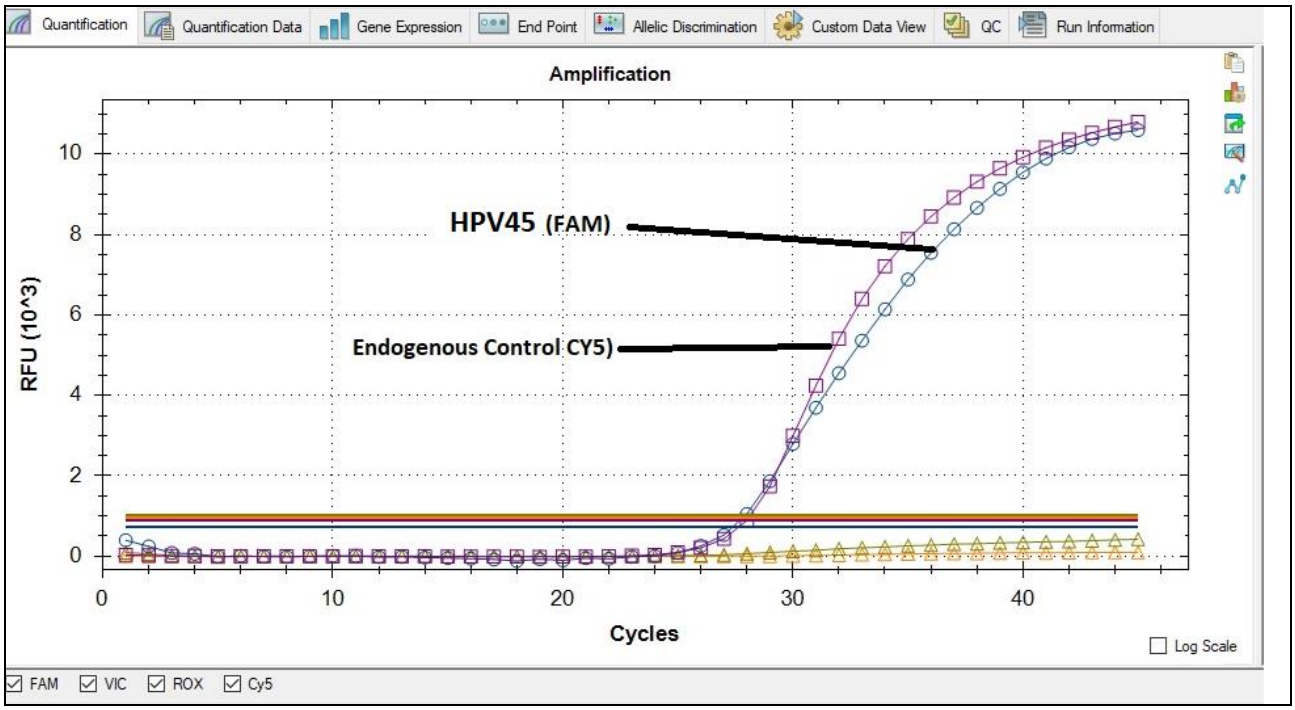
***If any of HPV type is positive, negative Endogenous Control can be omitted.**

	FAM	VIC	ROX	CY5	Interpretation
Mix 1	HPV 45 (-)	HPV 16 (-)	HPV 18 (-)	Endogenous Control (-)	Invalid*
Mix 2	HPV 31 (-)	HPV 35 (-)	HPV 33 (-)	HPV 39 (-)	
Mix 3	HPV 59 (-)	HPV 66 (-)	HPV 56 (-)	HPV 58 (-)	
Mix 4	HPV 51 (-)	HPV 52 (-)	HPV 68 (-)	HPV6/11 (-)	
Mix 5	HPV 40 (-)	HPV 42 (-)	HPV 61 (-)	HPV 26 (-)	
Mix 6	HPV 44 (-)	HPV 53 (-)	HPV 43 (-)	HPV 69 (-)	
Mix 7	HPV81/82 (-)	HPV 54 (-)	HPV 73 (-)	HPV 70 (-)	

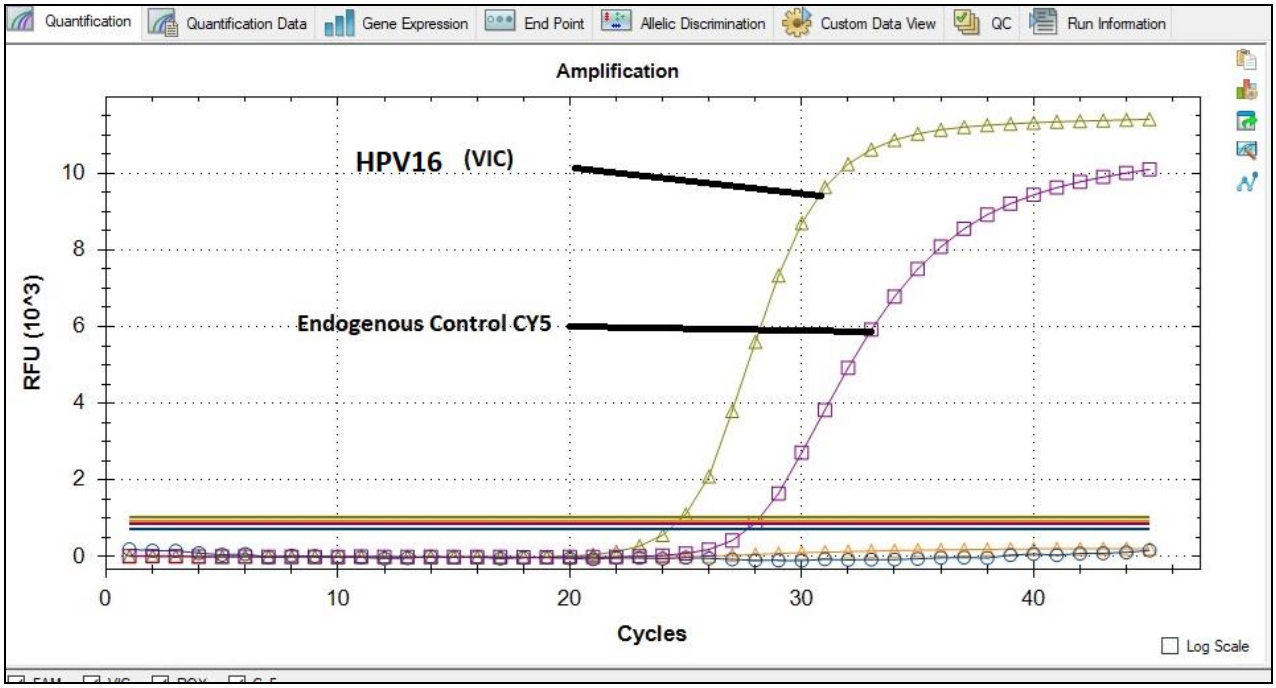
9. Results

Some Example Results.

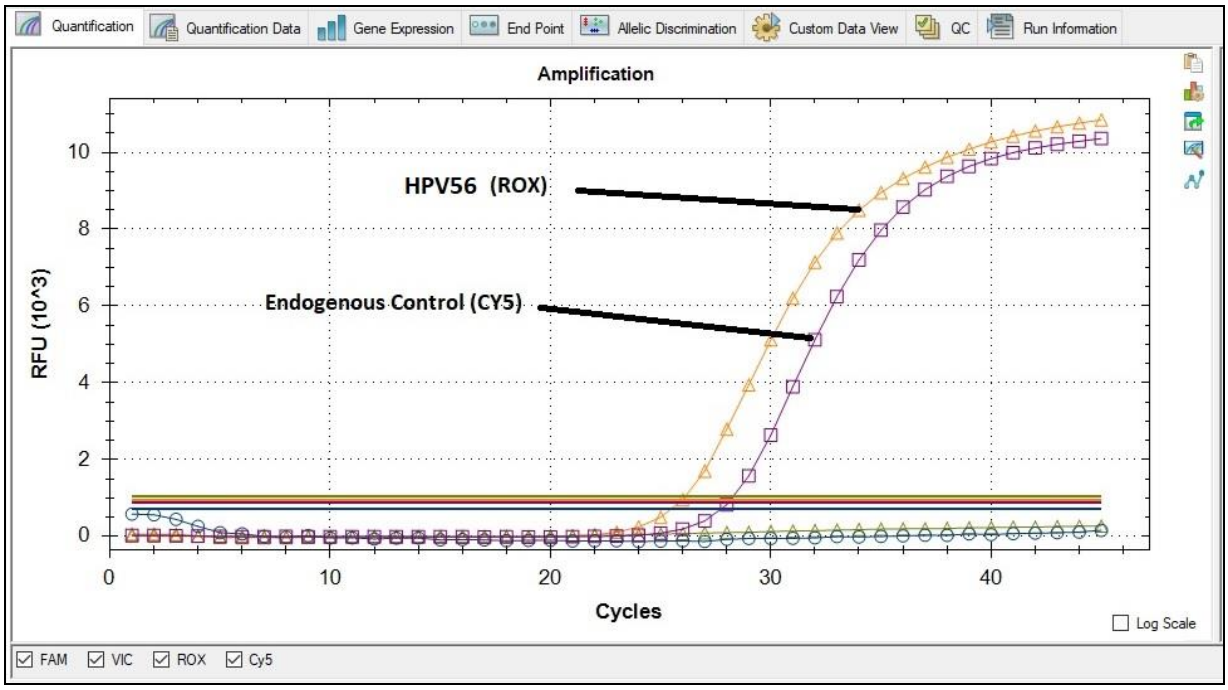
1-HPV45 Positive (Mix1)



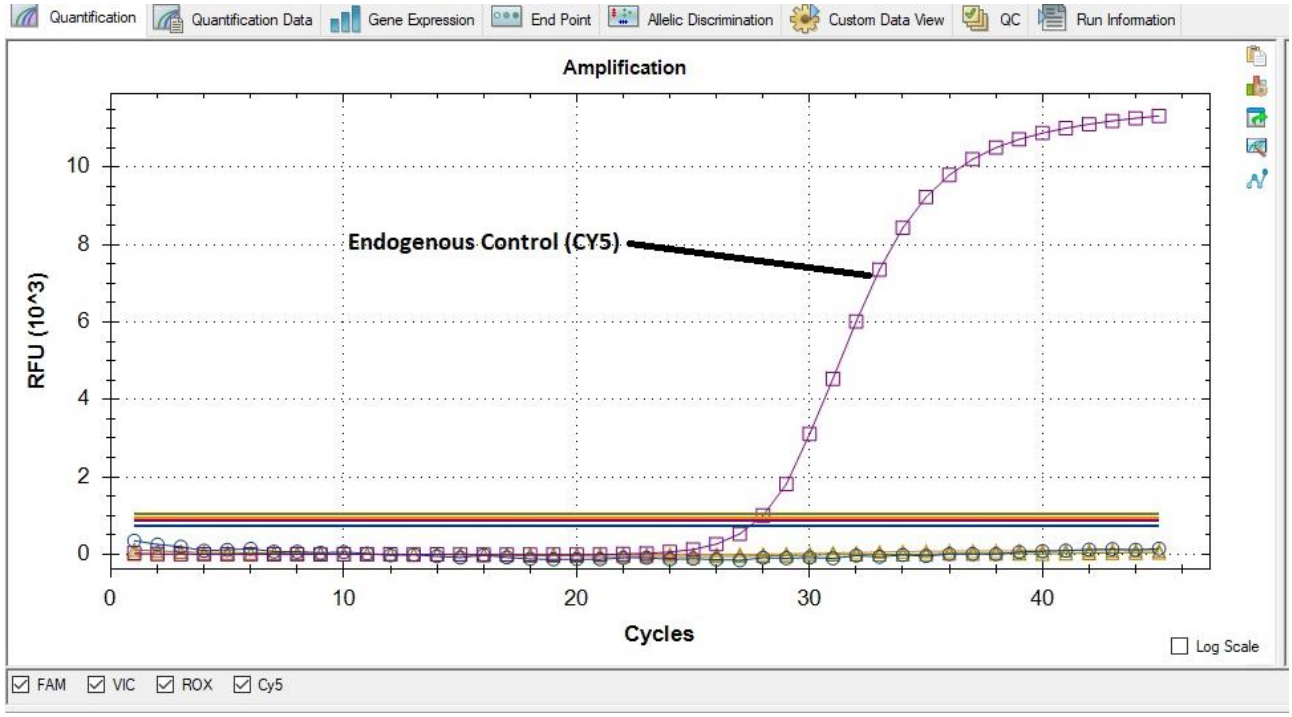
2-HPV16 Positive (Mix1)



3-HPV 56 Positive (Mix3)



4- Negative (Mix1)



10. Clinical Studies

The clinical performance of the geneMAP™ HPV 29 Genotyping Kit was established in one site clinical evaluation. Clinical specimens were tested with geneMAP™ HPV 29 Genotyping Kit and a CE-IVD approved commercial RT-PCR Kit as a comparator. Results are summarized below.

Note1: The instrument is Biorad® CFX96 Real-time PCR System / BIO-RAD

1- Cervical Swabs

Test Name	Comparator (CE-IVD Approved RT-PCR Kit) (HPV HR)			
geneMAP™ HPV 29 Genotyping Kit		Negative	Positive	**Total**
	Negative	698	6	**704**
	Positive	13	168	**181**
	Total	**711**	**174**	**885**

Statistic	Value	95% CI
Sensitivity	96.55%	92.65% to 98.72%
Specificity	98.17%	96.89% to 99.02%
Positive Predictive Value	92.82%	88.28% to 95.68%
Negative Predictive Value	99.15%	98.15% to 99.61%
Accuracy	97.85%	96.67% to 98.70%

2- FFPE (Head and Neck Cancer Biopsies)

Test Name	Comparator (CE-IVD Approved RT-PCR Kit) (HPV HR)			
geneMAP™ HPV 29 Genotyping Kit		Negative	Positive	**Total**
	Negative	50	0	**50**
	Positive	0	22 (HPV type 16)	**22**
	Total	**50**	**22**	**72**

Statistic	Value	95% CI
Sensitivity	100.00%	84.56% to 100.00%
Specificity	100.00%	93.51% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	100.00%	
Accuracy	100.00%	95.32% to 100.00%

HPV Types	Comparator (CE-IVD Approved RT-PCR Kit) Positive Sample Number	geneMAP™ HPV 29 Genotyping Kit Positive Samples Number
HPV16	28	28
HPV18	4	4
HPV45	4	5
HPV31	6	8
HPV66	15	14
HPV33	1	1
HPV39	4	4
HPV59	2	4
HPV35	2	2
HPV56	7	9
HPV58	2	2
HPV51	12	11
HPV52	11	9
HPV68	5	10
HPV6/11	32	32
HPV40	1	1
HPV42	10	10
HPV43	1	1
HPV44	3	3
HPV54	7	7
HPV61	2	2
HPV70	5	5
HPV26	1	1
HPV53	11	11
HPV68	12	12
HPV69	1	1
HPV73	2	2
HPV82	1	1
HPv 81	3	3
HPV Negative	261	261

11. Cross-Reactivity

In silico studies are summarized below (NCBI database);

NO.	Organism	In silico Analysis for % Identity targets
1	Chlamydia trachomatis	No alignment found
2	Chlamydia trachomatis (LGV I)	No alignment found
3	Chlamydia trachomatis (LGV II)	No alignment found
4	Chlamydia trachomatis (LGV III)	No alignment found
5	Chlamydia trachomatis (serovar A)	No alignment found
6	Chlamydia trachomatis (serovar B)	No alignment found
7	Chlamydia trachomatis (serovar Ba)	No alignment found
8	Chlamydia trachomatis (serovar C)	No alignment found
9	Chlamydia trachomatis (serovar D)	No alignment found
10	Chlamydia trachomatis (serovar E)	No alignment found
11	Chlamydia trachomatis (serovar F)	No alignment found
12	Chlamydia trachomatis (serovar G)	No alignment found
13	Chlamydia trachomatis (serovar H)	No alignment found
14	Chlamydia trachomatis (serovar I)	No alignment found
15	Chlamydia trachomatis (serovar J)	No alignment found
16	Chlamydia trachomatis (serovar K)	No alignment found
17	Mycoplasma genitalium	No alignment found
18	Mycoplasma hominis	No alignment found
19	Neisseria gonorrhoeae	No alignment found
20	Trichomonas vaginalis	No alignment found
21	Ureaplasma parvum	No alignment found
22	Ureaplasma urealyticum	No alignment found
23	Atopobium vaginae	No alignment found
24	Acinetobacter baumannii	No alignment found
25	Acinetobacter schindleri	No alignment found
26	Acinetobacter ursingii	No alignment found
27	Atopobium parvulum	No alignment found
28	Bacteroides caccae	No alignment found
29	Bacteroides fragilis	No alignment found
30	Bacteroides ovatus	No alignment found
31	Bacteroides vulgatus	No alignment found
32	Bacteroides xylanisolvens	No alignment found

33	Bifidobacterium adolescentis	No alignment found
34	Bifidobacterium longum	No alignment found
35	Bifidobacterium minimum	No alignment found
36	Candida albicans	No alignment found
37	Candida dubliniensis	No alignment found
38	Candida glabrata	No alignment found
39	Candida krusei	No alignment found
40	Candida lusitaniae	No alignment found
41	Candida orthopsilosis	No alignment found
42	Candida parapsilosis	No alignment found
43	Candida tropicalis	No alignment found
44	Candida metapsilosis	No alignment found
45	Chlamydophila pneumonidae	No alignment found
46	Chlamydophila psittaci	No alignment found
47	Clostridium difficile (Toxin A+ / B+)	No alignment found
48	Clostridium perfringens	No alignment found
49	Cytomegalovirus (CMV)	No alignment found
50	Enterococcus avium	No alignment found
51	Epstein Barr Virus	No alignment found
52	Escherichia coli	No alignment found
53	Gardnerella vaginalis	No alignment found
54	Haemophilus ducreyi	No alignment found
55	Haemophilus influenzae	No alignment found
56	Hepatitis A virus (HAV)	No alignment found
57	Hepatitis B virus (HBV)	No alignment found
58	Hepatitis C virus (HCV)	No alignment found
59	Human herpesvirus 1	No alignment found
60	Human herpesvirus 2	No alignment found
61	Human herpesvirus 3	No alignment found
62	Human Papilloma Virus 16	No alignment found
63	Human Papilloma Virus 18	No alignment found
64	Lactobacillus acidophilus	No alignment found
65	Lactobacillus amylovorus	No alignment found
66	Lactobacillus brevis	No alignment found
67	Lactobacillus casei	No alignment found
68	Lactobacillus crispatus	No alignment found

69	Lactobacillus delbrueckii subsp.	No alignment found
70	Lactobacillus fermentum	No alignment found
71	Lactobacillus fornicalis	No alignment found
72	Lactobacillus gallinarum	No alignment found
73	Lactobacillus gasseri	No alignment found
74	Lactobacillus helveticus	No alignment found
75	Lactobacillus iners	No alignment found
76	Lactobacillus intestinalis	No alignment found
77	Lactobacillus jensenii	No alignment found
78	Lactobacillus johnsonii	No alignment found
79	Lactobacillus kefiranofaciens	No alignment found
80	Lactobacillus oris	No alignment found
81	Lactobacillus parabuchneri	No alignment found
82	Lactobacillus pentosus	No alignment found
83	Lactobacillus plantarum	No alignment found
84	Lactobacillus reuteri	No alignment found
85	Lactobacillus rhamnosus	No alignment found
86	Lactobacillus salivarius subsp. Salicinius	No alignment found
87	Lactobacillus sanfrancisensis	No alignment found
88	Lactobacillus ultunensis	No alignment found
89	Lactobacillus vaginalis	No alignment found
90	Mobiluncus curtisii	No alignment found
91	Mobiluncus mulieris	No alignment found
92	Mycoplasma arginini	No alignment found
93	Mycoplasma felis Cole et al.	No alignment found
94	Mycoplasma iowae Jordan et al.	No alignment found
95	Mycoplasma leonicaptivi Hill	No alignment found
96	Mycoplasma pneumonia	No alignment found
97	Mycoplasma pulmonis	No alignment found
98	Mycoplasma spumans	No alignment found
99	Neisseria cinerea	No alignment found
100	Neisseria flavescens	No alignment found
101	Neisseria lactamica	No alignment found
102	Neisseria meningitidis	No alignment found
103	Neisseria mucosa	No alignment found
104	Neisseria perflava	No alignment found
105	Neisseria sicca	No alignment found
106	Neisseria subflava	No alignment found
107	Prevotella bivia	No alignment found

108	Prevotella buccalis	No alignment found
109	Prevotella disiens	No alignment found
110	Prevotella intermedia	No alignment found
111	Prevotella melaninogenica	No alignment found
112	Pseudomonas aeruginosa	No alignment found
115	Saccharomyces cerevisiae	No alignment found
116	Salmonella enteritidis	No alignment found
117	Salmonella typhimurium	No alignment found
118	Staphylococcus aureus	No alignment found
119	Streptococcus agalactiae	No alignment found
120	Streptococcus pneumoniae	No alignment found
121	Treponema pallidum	No alignment found
122	Vibrio parahaemolyticus	No alignment found
123	HPV1	No alignment found
124	HPV2	No alignment found
125	**HPV26**	**100% Match**
126	HPV34	No alignment found
127	**HPV40**	**100% Match**
128	**HPV42**	**100% Match**
129	**HPV43**	**100% Match**
130	**HPV44**	**100% Match**
131	**HPV53**	**100% Match**
132	**HPV54**	**100% Match**
133	**HPV61**	**100% Match**
134	HPV62	No alignment found
135	**HPV69**	**100% Match**
136	**HPV70**	**100% Match**
137	HPV71	No alignment found
138	HPV72	No alignment found
139	**HPV73**	**100% Match**
140	**HPV81**	**100% Match**
141	**HPV82**	**100% Match**
142	HPV83	No alignment found
143	HPV84	No alignment found
144	**HPV6**	**100% Match**
145	**HPV11**	**100% Match**
146	**HPV16**	**100% Match**
147	**HPV18**	**100% Match**
148	**HPV31**	**100% Match**
149	**HPV33**	**100% Match**
150	**HPV35**	**100% Match**
151	**HPV39**	**100% Match**
152	**HPV45**	**100% Match**

153	HPV51	**100% Match**
154	HPV52	**100% Match**
155	HPV56	**100% Match**
156	HPV58	**100% Match**
157	HPV59	**100% Match**
158	HPV66	**100% Match**
159	HPV68	**100% Match**

12. Limit of Detection

LoD Study has been performed by using spiked synthetic pathogen DNA's in the negative cervical swabs and results are summerized below;

Pathogen	LoD copies/reaction
HPV 16	20
HPV 18	25
HPV 45	30
HPV 31	20
HPV 35	25
HPV 33	30
HPV 39	25
HPV 59	30
HPV 66	25
HPV 56	40
HPV 58	50
HPV 51	35
HPV 52	25
HPV 68	20
HPV 6	30
HPV 11	20
HPV 26	20
HPV 40	25
HPV 42	30
HPV 43	20
HPV 44	50
HPV 53	40
HPV 54	25
HPV 61	20
HPV 69	30
HPV 70	25
HPV 73	40
HPV 81	35
HPV 82	25

13. Interfering substances

Interference testing was carried out using Human whole blood and cervical mucus as external materials not related with target species, geneMAP™ HPV 29 Genotyping Kit showed clear results that there is no influence on results observed under conditions mentioned above.

14. References

1) - https://www.who.int/news-room/fact-sheets/detail/cervical-cancer

2) -https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-and-cancer#:~:text=High%2Drisk%20HPVs%20can%20cause,for%20most%20HPV%2Drelated%20cancers.

15. Revision History

Date of Last Edit: December 2024		
Change	**Affected Section**	**Page**
Added reaction numbers for the kit	5.Reagents	7
Added ∑ (total numbers of tests) symbol	Cover Page	1
Fixed volume information of a reagent	5.Reagents	7
Updated Information	5.Reagents	7
Added Safety Instructions and General Warnings	2. Safety Instructions and General Warnings	3
Format updated	5.Reagents	7
Updated Information	8.2 Pretreatment of Specimens	9

16. Troubleshooting

geneMAP™ HPV 29 Genotyping Kit		
OBSERVATION	PROBABLE CAUSES	SOLUTION
No signal	The fluorophores for data analysis does not comply with the protocol	Select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition (See page 9) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Presence of inhibitor	Please dilute (1/10~1/100) the template nucleic acid with RNase-free Water and repeat the test with the diluted nucleic acid.
Putative false positive or target signals observed in Negative Control	Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips and throughout the procedure and change tips between tubes. Repeat entire procedure from nucleic acid extraction with the new set of reagents.
False negative or no signal observed in Positive Control	The fluorophores for data analysis does not comply with the protocol	Please select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect PCR mixture	Confirm that all components are added to the PCR mixture. Sensitivity is compromised with pre- composed premix. All reagents must be homogenized and spun down before use.
	Did not add sample's nucleic acid	Please carefully repeat the test.
	Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and carefully repeat the test if necessary.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition (See page 9) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure as well as nucleic acid concentration, and re-extract the nucleic acid. If the original specimen is not available, a new specimen must be collected.

17. Symbols Used

Catalog Number

Lot/Batch Number


Expiration Date

Storage Conditions


Manufactured by

Intended Use

18. Contact Information

Genmark Sağlık Ürünleri

İthalat İhracat ve Ticaret Limited Şirketi

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