MBPCR258 Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16,18 & 45 Multiplex) Probe PCR Kit

Instructions For Use

Description:
Human papillomavirus (HPV) is the most common sexually transmitted infection, caused by a double-stranded DNA virus with over 200 genotypes. While most infections are harmless, high-risk types like HPV 16, 18, and 45 are linked to cervical and other cancers. Globally, HPV-16 and HPV-18 together are responsible for approximately 70% of cervical cancer cases with HPV-16 accounting for about 60% of cases, HPV-18 about 15% of cases, and HPV-45 about 5% of cases. In India, cervical cancer is the second most common cancer in women aged 15–44, with HPV 16 being the most prevalent strain. HPV spreads mainly through sexual contact and infects the basal layer of epithelial cells. Risk factors include early sexual activity, multiple partners, smoking, poor hygiene, and weakened immunity. Real-time PCR (RT-PCR) is the preferred method for detecting and genotyping HPV. Vaccines targeting HPV 16 and 18 are available and play a key role in preventing cervical cancer.

NOTE: Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit is for *in-vitro* use only**.**

Intended Use:
The Hi-PCR® Human Papillomavirus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit is an in vitro diagnostic device intended for the qualitative detection and differentiation of HPV genotypes 16, 18, and 45 in clinical specimens. The kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Principle:
The Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit utilizes real-time PCR technology for the qualitative detection of HPV genotypes 16, 18, and 45. The assay employs hydrolysis probes labeled with a fluorescent reporter at the 5’ end and a quencher at the 3’ end, enabling specific and real-time detection of target DNA sequences. The kit detects the **L1 gene of HPV-16 (FAM)**, **HPV-18 (Texas Red)**, the **E6-E7 region of HPV-45 (JOE)** and **internal control (Cy5)** in a single-tube multiplex format. This multiplex design allows rapid and reliable HPV genotyping in clinical samples.

Controls:
Internal control (IC): An exogenous internal control, consisting of a non-human DNA sequence, is co-amplified in the same reaction tube using a distinct set of primers and probe. This control aids in detecting PCR inhibition and assessing the overall quality and reliability of the assay.

Positive control (PC): A positive control mimics a sample which contains all the target DNA sequences that the PCR is designed to amplify. It is included in a PCR assay to check the proper and intended functioning of all the PCR reagents.

ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED CE

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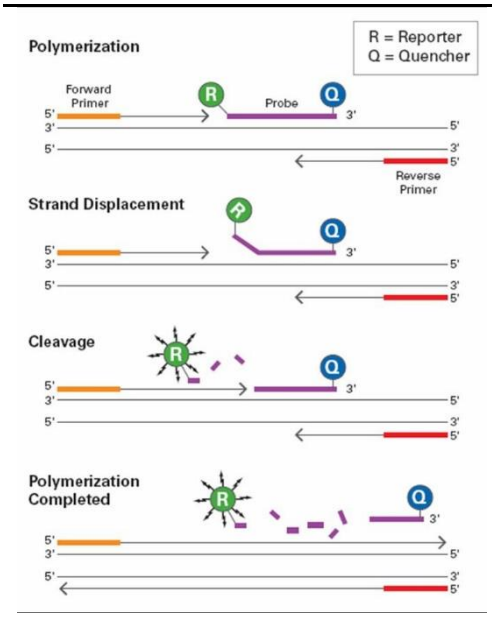
Negative control (NC): Using negative control mimics a PCR reaction that contains all the PCR reagents but does not include any DNA template. PCR grade water is used as the template to confirm that any observed amplification in the test samples is not due to contamination or non-specific amplification.

Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-Time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

Strand displacement: When the probe is intact, the report dye emission is quenched.

Cleavage: During each extension cycle,the DNA polymerae cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'- nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Features

Molecular Features:

- Simultaneous genotyping of high-risk HPV types 16, 18, and 45 in a single-tube multiplex assay.
- Capable of detecting low copy numbers of HPV DNA with high confidence.
- No cross-reactivity with common genital bacterial, fungal, or viral pathogens.

Technology features:

- Fast and reliable results within 75 minutes.
- Includes all reagents & controls for validity of the test.
- Open system – Compatible with 4, 5, and 6-channel qPCR cyclers.
- Wet-lab assays validated on the HiMedia's Insta Q96® series, Bio-Rad CFX Opus 96 and Applied Biosystems QuantStudio 5.

Sample Source: Cervical swabs, cervico-vaginal samples (women), anal swabs (men), and formalin-fixed paraffin-embedded (FFPE) tissue specimens.

Specimen collection and Handling

When handling specimens for HPV testing, always follow proper techniques to ensure safety and prevent contamination. All contaminated materials must be properly sterilized by autoclaving before disposal. Adhere to standard precautions as outlined in established guidelines for handling clinical specimens. This includes using appropriate personal protective equipment (PPE) and working in designated biosafety cabinets when necessary. For specific safety measures and disposal instructions, consult individual safety data sheets (SDS). It is essential to comply with all relevant safety protocols to minimize risk of exposure and ensure safe laboratory practices.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10°C and -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze-thaw cycle. Exposure to light, heat or humidity may also affect the shelf life of some of the kit components and should be avoided. Degradation of specimen/ extracted DNA can also hamper the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on pack.

Kit Contents: The provided PCR kit contains:

Components	Product Code	Reagents provided for (reactions)* (µL)	
		25R	50R
HPV 16,18 and 45 Multiplex Master Mix	DS2403	338	675
HPV Multiplex Primer-Probe Mix	DS1173	81	162
Internal Control Primer-Probe Mix	DS1168	27	54
Internal Control DNA	DS1022	27	54
HPV Multiplex Positive Control	DS1174	25	50
Molecular Biology Grade Water for PCR	ML065	75	150

***For a 25 µL PCR reaction**

Materials needed but not provided

All these materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and other equipment	
Insta Q96®AG Real time PCR System, 96 well block, 5 channels	MBLA027
Insta Q96®AG 6.0 Real time PCR System, 96 well block, 6 channels	MBLA028
Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012
TabSpin™ Microcentrifuge	LA1089/LA1090
Automated nucleic acid extraction systems	
Insta NX® Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX® Mag16, Insta NX® Mag16^{Plus}	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32^{Plus}	LA1096, MBLA019
Insta NX® Mag96	LA1097
Extraction Kits	
HiPurA® Viral DNA Purification Kit	MB575
HiPurA® Viral DNA/ RNA Purification Kit	MB582

HiPurA® Pre-filled Cartridges for Viral Nucleic Acid Purification	MB582PC16
HiPurA® Pre-filled Plates for Viral Nucleic Acid Purification	MB582MPF16
HiPurA® Pre- filled Plates for Viral Nucleic Acid Purification for Insta NX® Mag32	MB582MPF-32
HiPurA® Pre- filled Plates for Viral Nucleic Acid Purification for Insta NX® Mag96	MB582MPF-96
Tubes, plates and other consumables	
Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL)	LA611/LA614/LA615
µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)	LA955/LA958/LA959/LA960
Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL)	MBLA009/MBLA011/MBLA008
Barrier Tips, Maximum capacity 10 µL	LA749A
Barrier Tips, Maximum capacity 200 µL	LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859A
8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.1mL, 0.2 mL PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18

General Preparation Instructions

- Prior to use, ensure all PCR components are fully thawed on ice (4°C).
- Perform amplification reactions in a clean area, preferably within a biosafety cabinet.
- To minimize the risk of contamination from extraneous DNA templates, the use of aerosol barrier pipette tips is strongly recommended.
- If using a positive control sample, extract and store it separately from other reagents to prevent contamination. Add the positive control to the reaction mix in a designated, separate area.

Protocol for PCR Master Mix Preparation (For one reaction)

1. In the "Master Mix Preparation" area, thaw all kit components on ice. Mix by gently inverting the tubes, then centrifuge the reagents for 5 seconds. Keep the components on ice for later use.
2. Based on the number of specimens to be tested (N), calculate the required volume for each component by multiplying N by the volume of "1X" needed.
3. Use 1.5 mL nuclease-free centrifuge tube(s) to prepare the PCR reaction mix. Refer to the table below for the correct volumes. After adding all reagents, mix thoroughly and centrifuge for 5 seconds.

Components	**Volume (µL) to be added for 1X**
Preparation of PCR Reaction Mix	
Hi-Quanti 2X Realtime PCR Master Mix	12.5 µL
HPV Multiplex Primer-Probe Mix	3.0 µL
Internal control Primer-Probe Mix	1.0 µL
Internal Control DNA	1.0 µL
Molecular Biology Grade Water for PCR	2.5 µL
Total PCR Reaction Mix	**20.0 µL**
Template addition	
Test – Extracted sample viral DNA/ Positive Control – Provided in Kit/ Negative Template Control - Water	5.0 µL
Total volume	**25.0** µL

4. Aliquot 20.0 µL of the PCR reaction mix into the appropriately labeled 0.1/0.2 mL PCR tubes, plates, or strips, compatible with the PCR instrument being used.
5. In the designated "Nucleic Acid Handling" area, add 5.0 µL of extracted viral DNA from the test specimen to the respective wells of the plate/strip.
6. For the positive control and negative control tubes, replace the extracted sample viral DNA with the HPV Multiplex Positive Control (DS1174) and PCR-grade water (ML065), respectively.
7. Tightly cap the tubes/strips or seal the plate with an optically clear adhesive film.
8. Briefly centrifuge the tubes at 6000 rpm for approximately 10 seconds.
9. Place the tubes in the real-time PCR machine and set the recommended PCR program (outlined below).
10. Interpret the data from the amplification plot (observe the Ct values).

Recommended PCR program

1.	Initial denaturation	:	95°C for 10 minutes	
2.	Denaturation	:	95°C for 15 seconds	
3.	Annealing	:	55°C for 20 seconds (Plate Read)	No. of cycles: 40
	Channel	:	FAM/Texas Red/JOE/Cy5	

Data Analysis:

The following conditions should be met for a valid test:

Control	Target			
	HPV-16 (FAM)	HPV-18 (Texas Red)	HPV-45 (JOE)	Internal Control (Cy5)
Positive Control (PC)	+	+	+	+
Negative Control (NC)	-	-	-	+

Data Interpretation:

Detection Channel				Result Interpretation
HPV-16 (FAM)	HPV-18 (Texas Red)	HPV-45 (JOE)	Internal Control (Cy5)	
+	-	-	+*	Positive for HPV-16**
-	+	-	+*	Positive for HPV-18**
-	-	+	+*	Positive for HPV-45**
-	-	-	+	Negative for HPV-16, HPV-18 & HPV-45***
-	-	-	-	Possible PCR inhibition or extraction failure. Repeat PCR or re-extract and retest.

Ct value	Result
≤ 38	Detected (+)
> 38 or N/A	Not detected (-)

Kindly correlate the results with clinical findings.

A positive result should be interpreted in conjunction with clinical findings, such as cytology (Pap smear) or histopathology, to guide further diagnostic evaluation and patient management.

* In samples with high HPV DNA concentration, ***internal control amplification may be suppressed*** and is still considered a valid result if HPV is detected. However, we recommend test repetition using a freshly extracted sample.

** If sample is positive for two genotypes, re-test the specimen. If sample is repetitively positive for both the genotypes, the result may be indicative of a "***co-infection***".

Please note that co-infections (presence of more than one HPV genotype) are common, especially in Indian women with high-risk HPV infections. HPV 16 and HPV 18 are often found together in co-infections with other high-risk types like HPV 45, HPV 33, and HPV 31. These co-infection rates typically range from 20% to 40% among women with HPV infections based on data published by numerous studies in India.

** Few cases of co-infection of all three genotypes have been reported in the literature, ***however, re-testing the specimen along with confirmation with orthogonal test such as NGS is recommended***.

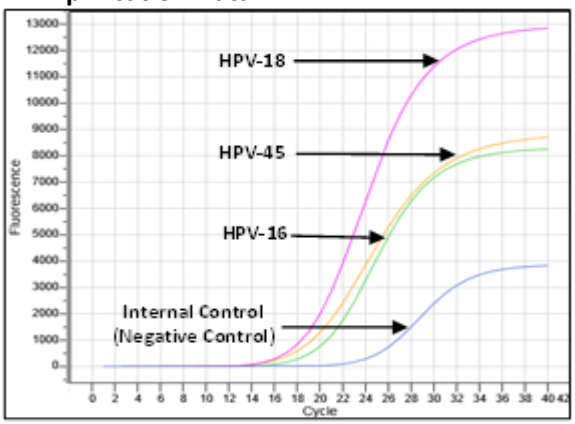
*** A negative result (no detection of HPV-16, 18, or 45) does not exclude HPV infection, as the assay is limited to these three genotypes. The patient may still be infected with other high-risk or low-risk HPV types, which this kit does not detect. Therefore, ***clinical findings and cytology must be considered***, and additional HPV testing may be recommended where broader genotyping is clinically indicated.

Visually inspect all amplification plots. Do not rely on Ct values alone. Please note that amplification curves exhibit a characteristic sigmoidal shape. Curves that are non-sigmoidal or/and show a sudden rise in fluorescence at very early Ct values (e.g., Ct <6) may be due to non-specific amplification, background noise, or instrument artifacts. Such results should be repeated or confirmed before making any clinical interpretation.

Quality Control

Every lot of Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18, & 45 Multiplex) Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality. The Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18, & 45 Multiplex) Probe PCR Kit provides controls- Positive Control and Negative Control which are to be included in every run.

Amplification Data



Sr. No	Sample	Ct value
1	HPV-16 (Positive Control)	20.77
2	HPV-18 (Positive Control)	19.43
3	HPV-45 (Positive Control)	18.26
3	Internal Control (Negative Control)	25.08

Representative image showing amplification plot of HPV-16, HPV-18, HPV-45 and Internal Control with Ct values, using Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16,18,45 Multiplex) Probe PCR Kit. The results may vary depending upon the sample types.

Performance Evaluation

Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit was conducted using Quantitative synthetic DNA from ATCC of Human Papillomavirus 16 (VR-

3240SD), Human Papillomavirus 18 (VR-3241SD) and Human Papillomavirus 45 synthetic DNA on InstaQ96® Real Time PCR systems. The detectable limit of the Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit on Real Time instrument was determined to be **~10 copies/reaction**.

Inclusivity - Analytical Sensitivity

In silico analysis for the assessment of inclusivity for the Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit was conducted by mapping the primers and probes against all the available Human Papilloma Virus (HPV) sequences in GenBank. The Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit targets 100% of the known HPV Genotypes 16, 18 and 45.

Cross-reactivity - Analytical Specificity

In silico analysis was performed using NCBI nucleotide and Primer BLAST. The primers for Human Papilloma Virus (HPV) 16, 18 and 45 were analyzed against bacteria, yeast and virus recommended for genital diseases (data not shown). The Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit was 100% specific for the known HPV Genotypes 16, 18 and 45.

Wet testing analysis was performed against the following pathogens mentioned below in the table. No cross-reaction was observed with any strains.

Acinetobacter anitratus	*Bacteroides vulgatus*
Chlamydia trachomatis	Mycoplasma spps.
Enterobacter cloacae	Saccharomycetales spps.
Salmonella enterica	Davidiellaceae spps.
Staphylococcus aureus	Cladosporium spps.
Candida albicans	Pichia spps.
Escherichia coli	*Lactobacillus crispatus*
Gardnerella vaginalis	*Lactobacillus jensenii*
Neisseria gonorrhoeae	*Monilial vaginitis*
Trichomonas vaginalis	Adenovirus
Enterrococcus faecalis	Hepatitis B virus
Haemophilus ducreyi	Hepatitis C virus
Treponema pallidum	Hepatitis E virus
Gardnerella spps.	Human Immunodeficiency virus
Prevotella spps.	Epstein-Barr virus
Ureaplasma spps.	Herpes Simplex virus
Bifidobacterium infantis	Simian virus

Warning and Precautions

Certified for *in-vitro* diagnostics. Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

- Strict compliance with the Instructions for Use is required for optimal results and the use of the kit is limited to staff qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures.

- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Viral DNA should be extracted from clinical sample using appropriate nucleic acid extraction method.
- Presence of PCR inhibitors and other interferences may lead to false negative or invalid results.
- Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in failure to detect the presence of pathogen.
- As with any diagnostic test, results of the Hi-PCR® Human Papillomavirus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.
- Hi-PCR® Human Papillomavirus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit cannot detect other high-risk HPV genotypes (apart from HPV-16,18,45) and all low-risk HPV genotypes.
- Performance of the kit in monitoring treatment of HPV infection has not been evaluated.

Kit compatibility with Real-Time PCR Systems:

The Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit uses fluorophores compatible with a range of Real-Time PCR systems. However, performance validation has been specifically conducted only on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5, and Insta Q96® Plus Real-Time PCR Systems. Below is the fluorophore compatibility matrix for commonly used instruments:

Real-Time PCR system	Company	HPV 16	HPV 18	HPV 45	Internal Control
Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4	HiMedia Laboratories Pvt. Ltd.	FAM	Texas Red	JOE	Cy5
QuantStudio™ 5	Applied Biosystems	FAM	ROX	VIC/JOE	Cy5
BioRad CFX Opus 96/CFX96 Touch	Bio-Rad Laboratories, Inc.	FAM	Texas Red	HEX/JOE	Cy5
ABI® Prism SDS 7500	Applied Biosystems	FAM	Texas Red	JOE	Cy5
Rotor-Gene®6000 & Q	QIAGEN	Green	Orange	Yellow	Red
Roche LightCycler® 96	Roche	FAM	Texas Red	HEX/JOE	Cy5
AriaMx	Agilent	FAM	ROX	HEX/JOE	Cy5

Note: Ensure that your instrument is calibrated for the specific dyes listed above and is maintained according to the manufacturer's guidelines. Inconsistent calibration or dye incompatibility may result in abnormal amplification plots or failed detections.

Evaluation

Each lot of Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Troubleshooting Guide

Sr. No.	Problem	Possible Cause	Solution
1.	No amplification in test and/or control wells	Degraded samples or poor-quality DNA template	Use freshly extracted, high-quality DNA. Check DNA concentration and purity (e.g., A260/280 ratio). Avoid repeated freeze-thaw cycles for stored DNA.

		Missing or incorrect addition of reagents	Verify all reagents were added in the correct volumes and order. Recheck reaction setup steps. Use a master mix to reduce pipetting errors.
		Incorrect thermal cycling conditions	Cross-check the PCR cycling profile with the IFU.
		Expired or improperly stored reagents	Confirm the expiry date and proper storage. Avoid using reagents that have undergone multiple freeze-thaw cycles
		Instrument malfunction	Verify that the real-time PCR instrument is functioning properly and calibrated.
2.	Variability between replicates	Inconsistent pipetting or error in reaction setup	Prepare a single master mix for all replicates to minimize variation. Vortex thoroughly and aliquot carefully. Use calibrated pipettes and consistent technique.
		Air bubbles in reaction mix	Briefly centrifuge PCR tubes or plate before placing in the instrument to eliminate air bubbles.
		Uneven mixing of reagents	Ensure all reagents are fully thawed and mixed by vortexing before use. Spin down before pipetting.
		Edge effect (thermal variation across plate)	Avoid using outer wells in PCR plates if not temperature-uniform; use a plate seal and consistent plate layout.
3.	Amplification in negative control	Reagent contamination	Replace all critical reagents. Clean workspace, pipettes, and repeat analysis with fresh aliquots. Use filter tips and maintain a unidirectional workflow.
		Cross-contamination or aerosol contamination during reaction setup	Maintain strict unidirectional workflow. Set up reactions in a PCR hood or clean bench. Use aerosol-resistant filter tips for all pipetting steps. Avoid fast, forceful pipetting. Regularly clean work surfaces and equipment with DNA-decontaminating agents. Minimize opening of positive control tubes and avoid splashing.
		Template contamination in workspace or pipettes	Decontaminate work surfaces, pipettes, and equipment using DNA/RNA decontamination solutions. Perform regular cleaning.
		Improper sealing of PCR plate/tubes	Ensure plates/tubes are properly sealed to prevent cross-well contamination during thermal cycling. Use optical-grade seals if required.
4.	No signal with positive control or partial target amplification	Degradation of the positive control material due to improper storage or repeated freeze-thaw cycles	Use a fresh aliquot of positive control. Ensure storage conditions follow IFU and avoid repeated freeze-thaw. Discard expired or compromised controls.

		Incorrect thermal cycling conditions	Cross-verify cycler settings with IFU. Ensure annealing/extension temperature and time are as specified.
		Improper mixing of PC before use	Vortex and briefly spin down the positive control before adding it to the PCR mix. Mix well to ensure homogeneity.
5.	Early Ct value with non-sigmoidal amplification	Instrumental noise, background fluorescence, or non-specific amplification	Visually inspect amplification plots. Disregard flat or atypical (non-sigmoidal) curves that show a sudden rise in fluorescence with very early Ct values (e.g., <6). These may not indicate true amplification. Repeat the assay if necessary. Adjust threshold setting in software within exponential phase. Refer to instrument-specific guidance.
6.	Non-specific amplification of targets in samples and/or negative control	Improper threshold setting in the real-time PCR software	Visually inspect all amplification plots. Do not rely on Ct values alone. Manually adjust the threshold above the baseline noise, ensuring it falls within the exponential phase of valid sigmoidal amplification curves. Avoid setting the threshold too low (e.g., in the baseline region), which may falsely pick up background noise as true signal. Refer to the instrument-specific guidelines or user manual for proper threshold adjustment.
		Contamination of reagents or workspace	Use fresh aliquots of reagents. Prepare reactions in a contamination-free environment using separate areas for pre- and post-PCR steps. Use aerosol-resistant filter tips and routinely decontaminate surfaces and pipettes.
7.	No Internal Control (IC) amplification in HPV-positive sample	High HPV viral load may suppress IC amplification	If valid Ct for HPV target is observed, it is still considered a valid result. Test repetition with freshly extracted sample is recommended.
8.	High Ct values in positive samples (late amplification)	Low viral load or suboptimal sample	Results near the cut-off should be interpreted with caution. Repeat test using freshly extracted DNA. Confirm with orthogonal test if clinically significant.
9.	Inconclusive result (no amplification of HPV or IC)	Sample inhibition, extraction failure, or expired reagents	Repeat extraction or test with fresh sample. Check IC amplification to rule out PCR inhibition. Use validated extraction method and fresh reagents.
		Instrument malfunction	Verify that the real-time PCR instrument is functioning properly and calibrated.
10.	Signal in only one replicate (of duplicate or triplicate reactions)	Pipetting error or borderline positivity	Repeat the test. If consistent upon retesting, interpret cautiously in context of clinical findings. Borderline cases (Ct of 37-38) may require repeat sampling or orthogonal testing (e.g., NGS). Use calibrated pipettes and proper technique.

Safety Information

Hi-PCR® Human Papilloma Virus (HPV) Genotyping (16, 18 & 45 Multiplex) Probe PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Disposal

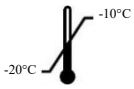
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures while disposing the infectious materials. Material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Please refer disclaimer Overleaf.

Symbols:

	Manufacturer		Do not use if package is damaged
	Authorized representative in the European Community		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		In vitro diagnostic medical device
	Batch code		CE marking of conformity
	Catalogue number		

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