MBPCR225 Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit

Description

Human papillomavirus (HPV) is the most common sexually transmitted infection, caused by a double-stranded DNA virus with over 200 genotypes. Genital HPV genotypes are classified into high-risk (oncogenic) and low-risk (non-oncogenic) types based on their potential to cause cancer. While low-risk HPVs cause benign conditions like warts, persistent infections with high-risk types can lead to precancerous lesions and certain cancers over many years. Though most infections are harmless, high-risk (HR) types like HPV16, HPV18, HPV 31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66 and HPV68 are linked to cervical and other cancers. Compared with conventional cervical cancer screening, molecular tests like PCR that specifically detect the presence of HR HPV DNA in cervical cells can potentially increase sensitivity and cost effectiveness of cervical cancer mass screening programs. Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit detects 14-HR HPV genotypes in a single tube assay with high sensitivity and specificity.

NOTE: Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit is for *in vitro* use only.

Intended Use:

Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit is a qualitative conventional PCR kit which results in specific amplification of target gene from Human Papilloma Virus HR genotypes.

Product Description:

Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit is designed to detect 14-HR HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) targeting either E6 or E7 or L1 gene of HPV genome. The kit also includes positive control to ensure the reliability and accuracy of the test results.

Principle

Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit is designed to detect 14-HR HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) targeting either E6 or E7 or L1 gene of HPV genome in a single tube format. The Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit operates on the principle of conventional PCR, a highly sensitive and specific method for gene amplification and detection.

The PCR process involves three key steps:

1. Denaturation: The double-stranded DNA is heated to a high temperature, causing the DNA to melt into two single strands.
2. Annealing: The reaction temperature is lowered, allowing the sequence-specific primers to bind to the target DNA sequences.
3. Extension: Taq DNA polymerase extends the primers by adding nucleotides to the single stranded DNA, resulting in the amplification of the target sequence.

These steps are repeated multiple times (typically 25 to 40 cycles) to exponentially amplify the target sequences. Following amplification, gel electrophoresis is used to analyze the PCR products. This technique separates DNA fragments based on size, allowing for the visualization and confirmation of the target gene's presence.

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

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Positive control

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.

Features

- Simultaneous detection of 14-HR HPV genotypes in a single assay
- Highly specific – No cross reactivity with pathogens with similar clinical presentation

Recommended Sample Types: Cervical swabs, Cervico-vaginal samples in women, Formalin fixed paraffin embedded (FFPE) tissue specimen.

Specimen collection and Handling

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with blood and other body fluids. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10°C to -20°C. Repeated thawing and freezing of PCR reagents should be avoided, not more than 5 freeze-thaw cycles, 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 and thaw. 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 reduce 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	100R
HR HPV Semi-Q Master Mix	DS2758	135	270	530
HR HPV Semi-Q Primer Mix	DS2759	54	108	212
HR HPV Semi-Q Positive Control	DS2760	54	108	212
Molecular Biology Grade Water for PCR	ML065	135	270	530
6X Gel Loading Buffer	ML015	54	108	212
50 bp DNA ladder	MBT084	15	30	60

*For a 20 µL PCR reaction

Materials needed but not provided:

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19)
- Thermal Cycler (Product Code: LA948/LA949/LA950/LA1006/LA1015/LA1059/LA1060/LA1066)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes

General Preparation Instructions

- Before use, suitable amount of all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.

- Extract and store positive control material (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.
- Centrifuge the components briefly once thawed.

A. Protocol for PCR Master Mix Preparation

Perform PCR reactions for each DNA sample as per the following table:

Components	Product code	Volume to be added for 1R (for a 20 µL reaction)
HR HPV Semi-Q Master Mix	DS2758	5
HR HPV Semi-Q Primer Mix	DS2759	2
Template DNA	-	10
Molecular Biology Grade Water for PCR	ML065	3

NOTE: i) For a Positive Control reaction tube, 10 µL of positive control DNA (DS2760) is to be added along with above mentioned components instead of template (extracted DNA).
ii) For a No template control reaction tube, 10 µL of Molecular Biology Grade Water for PCR (ML065) is to be added along with above mentioned components instead of template (extracted DNA).

Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below). Interpret the data using Agarose Gel Electrophoresis.

B. Recommended PCR program

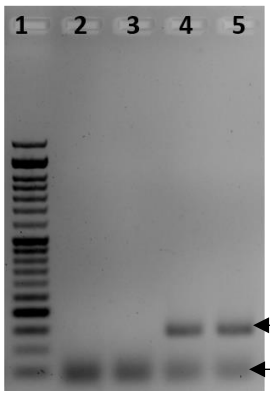
1	Initial denaturation	95°C for 10 minutes	No. of cycles: 1
2	Denaturation	95°C for 15 seconds	No. of cycles: 35
3	Annealing	60°C for 30 seconds	
4	Extension	72°C for 15 seconds	

C. After amplification, the products may be kept at 4°C overnight or frozen at –20°C for long-term storage.

D. PCR Assay Results Interpretation:
- For analysis of the PCR data, load 10 µL of amplicon on a 1.5% Agarose gel along with 1 µL of 6X Gel Loading Buffer (ML015).
- Load 3 µL of 50 bp DNA ladder (MBT084) in separate well.

E. EtBr-staining staining to check results:
- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 minutes.
- Confirm the expected amplicon size comparing with 50 bp DNA marker.

F. Amplification Data



Lane	Sample
Lane 1	50bp DNA ladder
Lane 2 and 3	No Template control
Lane 4 and 5	Positive control (150bp)

Gel image representing amplification of E6 or E7 or L1 gene of HPV genome (150-200bp)

A positive PCR result i.e. visible 150-200bp band in the sample indicates the presence of High-risk HPV genotype in the sample. 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.

A negative result (i.e. absence of 150-200bp band in clinical sample) does not exclude HPV infection, as the assay is limited to above mentioned 14-HR genotypes. The patient may still be infected with other less common 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.

Analytical Specificity

Inclusivity

The ability of the Hi-PCR® Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit to detect a wide range of related target organisms has been assessed in the inclusivity parameter by two ways (i) *in silico* analysis of the primers and (ii) wet lab testing using nucleic acids of related target organisms. The oligonucleotide sequences of all the targets were checked by sequence comparison against all the relevant sequences of HPV16, HPV18, HPV31, HPV33, HPV35, HPV 39, HPV45, HPV 51, HPV52, HPV 56, HPV58, HPV 59, HPV 66 and HPV 68 available in the GenBank database.

Inclusivity was further verified by wet lab testing of the primers against commercially available controls or standards for HPV16, 18, 31, 33, 45, 52, 58 using WHO International Standard Collection for Human Papillomavirus (HPV) DNA Genotypes- HPV16 (NIBSC code: 06/202), HPV18 (NIBSC code: 06/206), HPV31, HPV33, HPV45, HPV52, HPV58 (NIBSC code: 19/226).

Exclusivity / Cross-Reactivity Analysis

Wet lab testing of the Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit for potential cross-reactivity was performed using DNA/RNA from various pathogens available in the laboratory. None of the pathogens listed in the table below exhibited any reactivity with the primers of the Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit.

*Neisseria gonorrhoeae (*ATCC700825DQ)	Human herpesvirus 2 (ATCC VR-540DQ)
*Trichomonas vaginalis (*ATCC30001DQ)	Human immunodeficiency virus 1 (ATCC 3245SD)
*Mycoplasma genitalium (*ATCC33530DQ)	Human herpesvirus 5 (ATCC VR-538DQ)
*Chlamydia trachomatis (*ATCCVR-885DQ)	Human gamma herpesvirus 4 (ATCC VR-3247SD)
Treponema pallidum (ATCCBAA-2642SD)	Hepatitis B virus (ATCC 3232SD)
*Gardnerella vaginalis (*ATCC14019DQ)	Hepatitis C virus (ATCC 3233SD)
*Candida albicans (*ATCC10231DQ)	Zika virus (ATCC 3252SD)
Candida dubliniensis (ATCC646DQ)	Ebolavirus (BEI NR-318120
Human herpesvirus 1(ATCC VR-539DQ)	HPV 6 (NIBSC code: 14/256)
HPV 11 (NIBSC code: 14/1000)	

Quality Control

Each lot of Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. It has been functionally tested in DNA amplification assays. The Hi-PCR® HPV 14 High Risk Semi-Q PCR Kit provides reagents for controls: HR HPV Semi-Q Positive Control (PC) and a No Template Control (NTC) which are to be included in every run.

Precaution

Read the procedure carefully before starting the experiment. 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.

Performance and Evaluation

Each lot of Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	1. Check the integrity of DNA using agarose gel electrophoresis.
			2. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
2.	Variability between replicates	Error in reaction set-up	Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a PCR machine.
		Pipetting error	Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.
4.	No signal with positive controls	Incorrect programming of the temperature profile of the thermocycler	Compare the temperature profile to the manual

Safety Information

Hi-PCR® HPV 14-High Risk Semi-Q PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

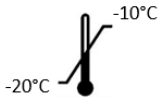
Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and 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.

Symbols

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number
	In vitro diagnostic medical device		

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