# MBPCR105 Hi-PCR® Human Papilloma Virus (HPV) Genotyping (Multiplex) Probe PCR Kit

**Description:**
Human papillomavirus (HPV) infection is caused by a human papillomavirus, a DNA virus from the papillomavirus family, of more than 200 related viruses and is the most common sexually transmitted infection (STI). While most HPV infections are benign causing warts (papillomas) on areas of the body including the hands, feet and genitals, certain strains put a person at a higher risk of developing particular types of cancers. HPV infection is limited to the basal cells of stratified epithelium, the only tissue in which they replicate. They typically spread by sustained direct skin-to-skin contact with oral, vaginal and anal sex being the most common methods. Risk factors for persistent HPV infections include early age at first sexual intercourse, multiple partners, smoking and poor immune function. Occasionally, it can spread from a mother to her baby during pregnancy. The high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 are known to cause several types of cancer. Among the high-risk HPV types identified, HPV types 16 and 18, are responsible for most HPV-caused cancers. Hence, the focus on these genotypes gives the physician useful details upon which to make effective treatment decisions. Real time PCR technique is considered simple and fast method for diagnosis with respect to the standard PCR technique. This technique has been successfully used for the rapid and accurate method for diagnosis of HPV infection in humans. Hi-PCR® Human Papilloma Virus (HPV) Genotyping (Multiplex) Probe PCR Kit detects and differentiate HPV 16 and 18 genotypes in a single tube multiplex assay with high sensitivity and specificity.

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

**Intended Use:**
Hi-PCR® Human Papilloma Virus (HPV) Genotyping (Multiplex) Probe PCR Kit is intended for use by qualified laboratory personnel trained in Real-Time PCR. The kit is recommended for sensitive and specific detection of HPV16 and HPV18 in clinical samples. The results from Hi-PCR Human Papilloma virus (HPV) Genotyping (Multiplex) Probe PCR kit must be interpreted within the context of all relevant clinical and laboratory findings.

**Principle:**
Real-time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. Hi-PCR® Human Papilloma Virus (HPV) Genotyping (Multiplex) Probe PCR Kit is designed to detect **the E6 region of HPV16 and HPV18 in FAM channel and Texas Red channel respectively, and an endogenous Internal Control in Cy5 channel** in a single tube reaction. The kit allows sensitive detection and differentiation of HPV16 and HPV18 in a single tube assay.

**Internal control (IC):** An endogenous internal control is a DNA sequence that is naturally present in the test samples and is amplified in the same tube with a different set of primers and probe which may aid in identification of possible PCR inhibition, DNA purification efficiency and assessing the quality of the sample.



Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com


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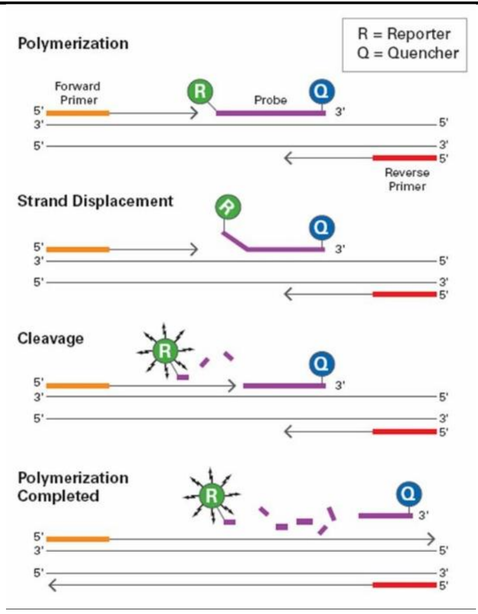
**Positive control (PC):** A positive control mimics a sample which contains all the target DNA sequences that the PCR is designed to amplify. It is included in a PCR assay to check the proper and intended functioning of all the PCR reagents.
**No template control (NTC):** A No Template Control is a 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 detection and differentiation of HPV16 and HPV18 in a single assay
- Highly sensitive – 2 IU/µL for both HPV16 and HPV 18
- Highly specific – No cross reactivity with pathogens with similar clinical presentation

**Technology features:**

- Fast and reliable results within 90 minutes.
- Includes all reagents & controls for validity of the test.
- Open system – Compatible with 4-channel and 5-channel qPCR cyclers.
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

**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 RNA 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 |
| HPV16-18 Master Mix | DS2285 | 135 | 270 | 530 |
| HPV16-18 Primer Probe Mix | DS2286 | 81 | 162 | 318 |
| HPV16-18 Positive Control | DS2301 | 54 | 108 | 212 |
| Molecular Biology Grade Water for PCR | DS0440 | 162 | 324 | 648 |

*For a 20 µL PCR reaction

**Materials needed but not provided**

All materials are available through www.himedialabs.com

| Product name | Product Code |
|---|---|
| **Real-Time PCR Instrument and equipment** | |
| | |
| Insta Q96® Real time PCR System, 96 well block, 5 channels | LA1012 |
| Insta Q48® M4 Real time PCR System, 96 well block, 4 channels | LA1023 |
| 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 |
| TabSpin™ Microcentrifuge | LA1089/LA1090 |
| **Automated nucleic acid extraction system and materials** | |
| 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 Paraffin-Embedded Tissue Nucleic Acid Purification | MB530MPF16 |
| HiPurA® Pre-filled Cartridges for Paraffin-Embedded Tissue Nucleic Acid Purification | MB530PC16 |
| HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit (Cartridges) | MB583PC16200 |

| | |
|---|---|
| HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit (Plates) | MB583MPF16200 |
| HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit | MB583MPF32200 |
| HiPurA® Pre-filled Clinical Multi-purpose Magnetic Nucleic Acid Purification kit | MB583MPF96200 |
| **Tubes, plates and other consumables** | |
| Varivol II Micropipette-10 (Capacity: 0.5 to 10 µl) | LA611 |
| Varivol II Micropipette-100 (Capacity: 10 to 100 µl) | LA615 |
| Varivol II Micropipette-1000 (Capacity: 200 to 1000 µl) | LA614 |
| Barrier Tips, Maximum capacity 10 µl | LA749/LA749A |
| Barrier Tips, Maximum capacity 200 µl | LA751/LA751A |
| Barrier Tips, Maximum capacity 1000 µl | LA859/LA859A |
| 8-strip tubes & optically clear flat caps for PCR | PR17, PR22, PR23 |
| PCR Tubes, 0.2 mL; PCR Plates | PW1255/ PR2/PR3/PR19 |
| Optical Sealing film | PR18 |

**Kit compatibility with Real-Time PCR Systems**

The Hi-PCR® Human Papilloma Virus (HPV) Genotyping (Multiplex) Probe PCR Kit contains fluorophores compatible with following PCR systems. However, its performance has been specifically validated on the Bio-Rad (CFX Opus 96), Applied Biosystems (QuantStudio 5), Insta Q96® AG and Insta Q96® Plus Real-Time PCR Systems. Below is the fluorophore compatibility matrix for commonly used instruments:

| Real-Time PCR system | Company | Dye 1 | Dye 2 | Dye 3 | Dye 4 |
|---|---|---|---|---|---|
| Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4 | HiMedia Laboratories Pvt. Ltd. | FAM | JOE/HEX | Texas Red | Cy5 |
| BioRad CFX Opus 96/CFX96 Touch/ CFX384 Touch | Bio-Rad Laboratories, Inc. | FAM | JOE/HEX | Texas Red | Cy5 |
| QuantStudio™ 5 | Applied Biosystems | FAM | JOE/HEX/VIC | Texas Red/ ROX | Cy5 |
| ABI® Prism SDS 7500 | Applied Biosystems | FAM | JOE/HEX/VIC | Texas Red/ ROX | Cy5 |
| QIAquant 96 & 384 5plex | QIAGEN | FAM | JOE/HEX | Texas Red | Cy5 |
| Rotor-Gene®6000 & Q | QIAGEN | Green | Yellow | Orange | Red |
| LightCycler® 96 /LightCycler® 480 | Roche | FAM | JOE/HEX/VIC | ROX/ Texas Red | Cy5 |
| qTOWER$^3$ | Analytik Jena | FAM | JOE/HEX/VIC | ROX/ Texas Red | 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.**

**Warning and Precautions**

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.

**General Preparation Instructions**

- Before use, all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

**Protocol for PCR Master Mix Preparation (For one reaction)**

1. In the "Master mix Preparation" area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for several seconds. Keep on ice for later use.
2. Based on the number of specimens to be tested (N), including the PTC and NC, calculate the volume of the components to be added as N* volume of 1X.
3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the reaction system. After all the reagents are added, mix them thoroughly and centrifuge for several seconds.

| **Components** | **Product code** | **Volume for "1X" (one reaction)** |
|---|---|---|
| **Preparation of PCR Reaction Mix** | | |
| HPV16-18 Master Mix | DS2285 | 5.0 µL |
| HPV16-18 Primer Probe Mix | DS2286 | 3.0 µL |
| Water (PCR grade) | DS0440 | 2.0 µL |
| **Total PCR Reaction Mix** | - | 10.0 µL |
| **Template addition** | | |
| Template/ Purified Viral DNA | - | 10.0 µL |
| **Total reaction volume** | - | 20.0 µL |

4. Load 10µL of master mix into the 0.1/0.2 mL PCR reaction tube/plate/strips, compatible to the instrument to be used; add 10 µL of master mixture with 10 µL Nuclease free water to the negative control.
5. In the "Nucleic acid handling" area add 10 µL HPV Multiplex Positive Control and extracted test DNA into the plate/strip.
6. For positive control and no template control tube, Template Bacterial DNA is replaced by MTB/NTM Positive Control and by PCR grade water respectively. Refer the following table.

| **Set up of controls for the PCR run** | | |
|---|---|---|
| **Components** | **Product code** | **Volume for "1X" (one reaction)** |
| Total PCR Reaction Mix | - | 10.0 µL |
| Positive Control | | |
| HPV16-18 Positive Control | DS2301 | 10.0 µL |
| No Template Control | | |
| Water (PCR grade) | DS0440 | 10.0 µL |
| **Total reaction volume** | - | 20.0 µL |

7. Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film.
8. Briefly, spin the strips/tubes to settle the reagent to the bottom of the tube.

Place the plate/strips/tubes in Real-time PCR machine and set the PCR program. Interpret the data from the amplification plot (observe the Ct values).

| Sr. No | Step | Temperature | Time | Sampling | No. of cycles |
|---|---|---|---|---|---|
| 1. | Initial denaturation | 95°C | 10 minutes | --- | 1 |
| 2. | Denaturation | 95°C | 15 seconds | --- | 45 |
| 3. | Annealing | 60°C | 30 seconds | YES | |
| 4. | Extension | 72°C | 15 seconds | -- | |

**Selection of channels**

| Target | Channels | Quencher |
|---|---|---|
| HPV16 | FAM | None |
| HPV18 | Texas Red/ROX | None |
| Internal Control (IC) | Cy5 | None |

**Please select 'Passive reference dye' as 'None' wherever applicable**

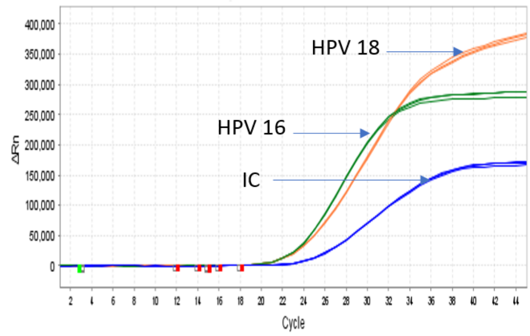
**Data Analysis**

The following conditions should be met for a valid test:

| Control | Target | | |
|---|---|---|---|
| | HPV-16 (FAM) | HPV-18 (Texas Red) | Internal Control (Cy5) |
| Positive Control (PC) | + | + | + |
| No Template Control (NTC) | - | - | - |

| Target | Ct value | Result |
|---|---|---|
| HPV16 | ≤ 34 | Detected (+) |
| HPV18 | ≤ 34 | Detected (+) |

**Amplification Data**




| Sr. No | PC | Ct value | |
|---|---|---|---|
| | | PC | NTC |
| 1 | HPV16 | 24 | - |
| 2 | HPV18 | 23 | - |
| 3 | IC | 26 | - |

**Note: Representative image showing amplification plot of HPV-16, HPV-18 and Internal Control with Ct values, using Hi-PCR® Human Papilloma Virus (HPV) Genotyping (Multiplex) Probe PCR Kit on Applied Biosystems QuantStudio™ 5 System. The results completely depend upon sample types.**

**Data Interpretation:**

| Detection Channel | | | Result Interpretation |
|---|---|---|---|
| **HPV-16 (FAM)** | **HPV-18 (Texas Red/ROX))** | **Internal Control (Cy5)** | |
| + | - | +/-* | Positive for HPV16 |
| - | + | +/-* | Positive for HPV18 |
| + | + | +/-* | HPV16 and HPV18 Coinfection |
| - | - | + | Negative for HPV16 & HPV18 |
| - | - | - | Inconclusive test** |

*The presence or absence of a signal in the Cy5 channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.
** When an inconclusive result is obtained, repeat PCR or re-test the extracted DNA or re-extract the specimen and test the newly extracted DNA

**Note:**

1) 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.
2) 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***".
3) A negative result (no detection of HPV16 and HPV 18 genotypes) does not exclude HPV infection, as the assay is limited to these genotypes. The patient may still be infected with other 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.
4) 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.

**Performance Evaluation**

**Limit of Detection (LoD) - Analytical Sensitivity**

The Limit of Detection (LoD) is defined as the concentration (copies per µl of the eluate) of target molecule that can be detected at 95% or greater probability according to CLSI EP17-A2. Sensitivity for the Hi-PCR® Human Papilloma Virus (HPV) Genotyping (Multiplex) Probe PCR Kit was conducted using 20 replicates of dilution series of known copy number of WHO International Standard Collection of Human Papillomavirus (HPV) DNA Genotypes HPV16 (NIBSC code: 06/202) and HPV18 (NIBSC code: 06/206), each on Biorad CFX Opus 96, Applied Biosystems QuantStudio 5, Insta Q96® AG and Insta Q96® Plus Real Time PCR Systems. The detectable limit of the Hi-PCR® Human Papilloma Virus (HPV) Genotyping (Multiplex) Probe PCR Kit on Real Time instrument was determined to be 2 IU/µl for both HPV16 and HPV 18.

**Inclusivity - Analytical Sensitivity**

*In silico* analysis for the assessment of inclusivity for the Hi-PCR® Human Papilloma Virus (HPV) Genotyping (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 (Multiplex) Probe PCR Kit targets 100% of the known Hi-PCR® Human Papilloma Virus (HPV) Genotyping 16 and 18.

**Specificity**

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

| Pathogen name | Name of Genomic standards used for the study | Catalog No. |
|---|---|---|
| *Neisseria gonorrhoeae* | Quantitative Genomic DNA from Neisseria gonorrhoeae | ATCC 700825DQ |
| *Trichomonas vaginalis* | Quantitative Genomic DNA from Trichomonas vaginalis strain C-1: NIH | ATCC 30001DQ |
| *Mycoplasma genitalium* | Quantitative Genomic DNA from Mycoplasma genitalium strain G37 | ATCC33530DQ |
| *Chlamydia trachomatis* | Quantitative Genomic DNA from Chlamydia trachomatis Serovar D strain UW-3/Cx | ATCCVR-885DQ |
| *Treponema pallidum* | Quantitative Synthetic Treponema pallidum DNA | ATCCBAA-2642SD |
| *Gardnerella vaginalis* | Quantitative Genomic DNA from Gardnerella vaginalis | ATCC 14019DQ |
| Human adenovirus | Quantitative Genomic DNA from Human adenovirus 1 strain Adenoid 71 | ATCCVR-1DQ |
| *Candida dubliniensis* | Quantitative Genomic DNA from Candida dubliniensis strain CBS 7987 | ATCC 646DQ |
| *Neisseria meningitidis* | Quantitative Genomic DNA from Neisseria meningitidis strain FAM18 | ATCC 700532DQ |
| *Candida albicans* | Quantitative genomic DNA from Candida albicans strain 3147 | ATCC10231DQ |
| | Quantitative genomic DNA from Candida albicans strain SC5314 | ATCC 2876DQ |
| Human papillomavirus 31 | WHO International Standards for Human Papillomavirus (HPV) DNA genotypes HPV31, HPV33, HPV45, HPV52, HPV58 | NIBSC 19/226 |
| Human papillomavirus 45 | | |
| Human papillomavirus 33 | | |
| Human papillomavirus 52 | | |
| Human papillomavirus 58 | | |
| Human herpesvirus 3 | Quantitative Genomic DNA from Human herpesvirus 3 (HHV-3) strain Ellen | ATCC 1367DQ |
| Human herpesvirus 5 | Quantitative Genomic DNA from Human herpesvirus 5 (HHV-5) strain AD-169 | ATCC 538DQ |
| Hepatitis B virus | Quantitative Synthetic DNA from Hepatitis B virus | ATCC 3232SD |
| Zika virus | Quantitative Synthetic RNA from Zika virus | ATCC 3252SD |
| Human immunodeficiency virus 1 | Quantitative Synthetic Human immunodeficiency virus 1 (HIV-1) RNA | ATCC 3245SD |
| Human gammaherpesvirus 4 | Quantitative Synthetic DNA from Human gammaherpesvirus 4 | ATCC 3247SD |
| Human herpesvirus 1 | Quantitative Genomic DNA from Human herpesvirus 1 | ATCC 539DQ |
| Human herpesvirus 2 | Quantitative Genomic DNA from Human herpesvirus 2 | ATCC 540DQ |

| | | |
|---|---|---|
| Hepatitis C virus | Quantitative Synthetic RNA from Hepatitis C virus | ATCC 3233SD |
| Ebolavirus | RNA from Bundibugyo Ebolavirus, Prototype Isolate #811250 (200706291 Uganda) | BEI NR-31812 |

**Cross-Reactivity Analysis – *in silico***

The oligonucleotide sequences (primers and probes) used in the Hi-PCR® Human Papilloma Virus (HPV) Genotyping (Multiplex) Probe PCR Kit were subjected to BLAST (Basic Local Alignment Search Tool) analysis against the organisms listed in the table below. No significant cross-reactivity was observed for any of the sequences during the in-silico BLAST analysis.

| Pathogen | Taxonomy | Accession No. |
|---|---|---|
| *Haemophilus ducreyi* | [Haemophilus] ducreyi strain VAN2 chromosome, complete genome | NZ_CP015425.1 |
| | [Haemophilus] ducreyi strain FDAARGOS_297 chromosome, complete genome | NZ_CP022037.2 |
| Human Simplex virus 1 | Human herpesvirus 1 strain 17, complete genome | NC_001806.2 |
| Human Simplex virus 2 | Human herpesvirus 2 strain HG52, complete genome | NC_001798.2 |
| Human herpesvirus 6 | Human betaherpesvirus 6A, variant A DNA, complete virion genome, isolate U1102 | NC_001664.4 |
| Group B Streptococcus (GBS) | Streptococcus sp. 'group B' strain FDAARGOS_229 chromosome, complete genome | NZ_CP020432.1 |
| *Ureaplasma urealyticum* | Ureaplasma urealyticum strain 132 chromosome, complete genome | NZ_CP041200.1 |
| | Ureaplasma urealyticum strain MIN-201 chromosome, complete genome | NZ_CP086123.1 |
| *Ureaplasma parvum* | Ureaplasma parvum strain hebnu3h04 chromosome, complete genome | NZ_CP021987.1 |
| | Ureaplasma parvum serovar 3 str. ATCC 700970, complete sequence | NC_002162.1 |
| *Mycoplasma hominis* | Metamycoplasma hominis ATCC 23114, complete sequence | NC_013511.1 |
| | Metamycoplasma hominis ATCC 27545 strain LBD-4 chromosome, complete genome | NZ_CP009652.1 |
| HPV 6 | Human papillomavirus type 6b, complete genome | NC_001355.1 |
| HPV 11 | Human papillomavirus type 11 (HPV-11) complete genome | NC_075235.1 |
| HPV 40 | Human papillomavirus type 7 genomic DNA | NC_001595.1 |
| HPV 42 | Human papillomavirus type 42 isolate TJ43-42, complete genome | GQ472847.1 |
| HPV 43 | Human papillomavirus type 43 complete genome | AJ620205.1 |
| HPV 44 | Human papillomavirus type 44, complete genome | NC_075252.1 |
| HPV 54 | Human papillomavirus 54, complete genome | NC_001676.1 |

| HPV 61 | Human papillomavirus type<br>61 isolate Qv3721, complete genome | KF436857.1 |
|---|---|---|
| HPV 30 | Human papillomavirus type 30 genomic DNA | NC_038889.1 |
| Human papillomavirus | Human papillomavirus isolate SE379, complete genome | NC_027779.1 |
| HPV 39 | Human papillomavirus ORFs | NC_075238.1 |
| HPV 35 | Human papillomavirus type 35 isolate QV29782, complete genome | HQ537729.1 |
| HPV 51 | Human papillomavirus 51 isolate BF315, complete genome | KF436887.1 |
| | Human papillomavirus 51 isolate 51YN25, complete genome | OR997941.1 |
| HPV 56 | Human papillomavirus type 56 genomic DNA | NC_075271.1 |
| HPV 59 | Human papilloma virus type 59, complete viral genome | NC_075272.1 |
| HPV 66 | Human papillomavirus type 66, complete genome | NC_075253.1 |
| HPV 68 | Human papillomavirus type 68a, complete genome | NC_075157.1 |
| HPV 31 | Human papillomavirus type 31 (HPV-31) complete genome | NC_075191.1 |
| HPV 33 | Human papillomavirus type 33, complete genome | NC_075233.1 |
| HPV 45 | Human papillomavirus type 45 genomic DNA | NC_075269.1 |
| HPV 52 | Human papillomavirus type 52 genomic DNA | NC_075270.1 |
| HPV 58 | Human papillomavirus type 58 complete genome | D90400.1 |
| | Human<br>papillomavirus 58 isolate ZWE054176, complete genome | KY225961.1 |

**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 Papilloma Virus (HPV) HR Genotyping Probe PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.
- Hi-PCR® Human Papilloma Virus (HPV) Genotyping (Multiplex) Probe PCR Kit cannot detect other common high-risk HPV genotypes and all low-risk HPV genotypes.
- Performance of the kit in monitoring treatment of HPV infection has not been evaluated.

**Evaluation**

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

**Quality Control**

Each lot of Hi-PCR® Human Papilloma Virus (HPV) Genotyping (Multiplex) Probe PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in amplification.

**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. 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. |
| | | Incorrect fluorophore/dye setting | Refer the selection of channels table and set the correct fluorophore in the real-time PCR instrument |
| | | 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. |

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| | | | 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 as recommended in the threshold setting table 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 | 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. |
| | | Improper / insufficient sample | If no signals from any of the HPV targets and also from the IC was observed, this indicates improper or insufficient sample collection. Repeat the sample collection. |

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| | | Presence of PCR inhibitor | Dilute the extracted nucleic acid in molecular biology grade water and repeat the PCR assay. |
| | | 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. |
| 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. | 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 (close to Ct cutoff) 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 (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

**Symbols**

| Symbol | Description | Symbol | Description |
|---|---|---|---|
|  | Manufacturer |  | Do not use if package is damaged |
| LOT | Batch code | -10°C<br>-20°C | Temperature limit |
|  | Date of manufacture (YYYY-MM) |  | Consult instructions for use |
|  | Use-by date (YYYY-MM) | REF | Catalogue number |
| IVD | In vitro diagnostic medical device |  |  |

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HiMedia Laboratories Pvt. Ltd. Reg.office : Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane, (West) 400604, Maharashtra, INDIA. Customer Care No.: 00-91-22-6116 9797 Tel: 00-91-22-6147 1919, 6903 4800 Email:mb@himedialabs.com Website: www.himedialabs.com