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Uterine Cervix Cancer of High-risk HPV Genotype Related Real Time PCR Kit User Manual

LT041300DT50

For use with ABI Prism®7500;Bio-Rad CFX96;SLAN-96P;LineGene9600; LightCycler®480 Instrument

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1. Intended Use

Uterine Cervix Cancer of High-risk HPV Genotype Related Real-time PCR kit is used for the detection of 13 Types of High-risk HPV Genotypes in genital swabs, cervical cells and paraffin section samples by using real time PCR systems.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

The human papilloma virus (HPV) is one of the most common virus groups in the world to affect the skin and mucosal areas of the body. Different types of the HPV's are known to infect different parts of the body. 13 types of genital tract HPV in particular, HPV 16, 18, 31,33,35,39,45,51,52,56,58,59, 68 are known to cause up to 99% of cervical cancers, and new studies show that they may be linked to oral cancer as well. All of these are genital viruses which are spread through sexual contact uterine cervix cancer of high-risk HPV genotype related real-time PCR kit contains a specific ready-to-use system for the detection of 13 Types of high-risk HPV genotypes by polymerase chain reaction in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of HPV DNA. Fluorescence is emitted and measured by the real time systems' optical unit during PCR. The detection of amplified HPV DNA fragment is performed in fluorimeter **channels FAM, HEX/VIC/JOE, TEXAS RED/Cal Red 610 and CY5** with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and genital swabs samples are used for DNA extraction. An external positive control contained all 13 types of high risk HPV partial sequence.

4. Kit Contents

Ref.	Type of Reagent	Presentation	50rxns
1	DNA Extraction Buffer	1 vial, 1.8ml	x2
2	HPV 16,56 ,31,IC Reaction Mix	1 vial, 950µl	x2
3	HPV 18,52,58,68 Reaction Mix	1 vial, 950µl	x2
4	HPV 35,45,33 Reaction Mix	1 vial, 950µl	x2
5	HPV 39,51,59 Reaction Mix	1 vial, 950µl	x2
6	PCR Enzyme Mix	1 vial, 42µl	x2
7	Molecular Grade Water	1 vial, 400µl	x2
8	HPV Positive Control	1 vial, 100µl	x2

Analysis sensitivity: 1×10^4 copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as it may reduce the assay sensitivity.
- Cool all reagents during the working steps.
- Reaction Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Vortex mixer
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 µl - 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and freezer
- Tube racks

7. ⚠ Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols.

8. Sample Collection, Storage and Transport

- Collect samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

9. Procedure

9.1 DNA-Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use. You may use your own extraction systems or commercial kits.

9.1.1 Genital swabs , cervical cells samples

1) Wash the sample in 1.0ml normal saline and vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 1.0ml normal saline and suspend the pellet with vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
3) Add 50µl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.
4) Incubate the tube for 10 minutes at 100°C.
5) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.1.2 Paraffin section samples

DNA extraction Buffer in the kit can't be used to extract DNA from paraffin section samples, but a commercial extraction kit is recommended: QIAamp DNA FFPE Tissue Kit(50)(Cat.No.56404)

Attention:

A. During the incubation, make sure the tube is not open. Since the vapor will volatilize into the air and may cause contamination if the sample is positive.
B. The extraction sample should be used in 3 hours or stored at -20°C for one month.
C. DNA extraction kits are available from various manufacturers. You can also use your own extraction systems or the commercial kit depending on the yield. For DNA extraction, please comply with the manufacturer's instructions.

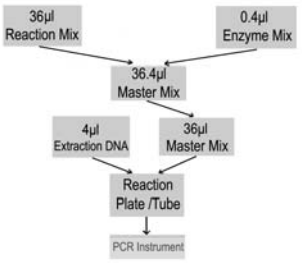
9.2 Internal control

MNBH gene is detected as an internal control. All clinical samples should exhibit MNBH positive, thus indicating the presence of sufficient nucleic acid from human MNBH gene. Failure to detect MNBH in any of clinical samples may indicate that:

1) Improper extraction of nucleic acid.
2) Absence of sufficient human cellular material in sample.
3) Improper assay set up and execution
4) Reagent or equipment malfunction.

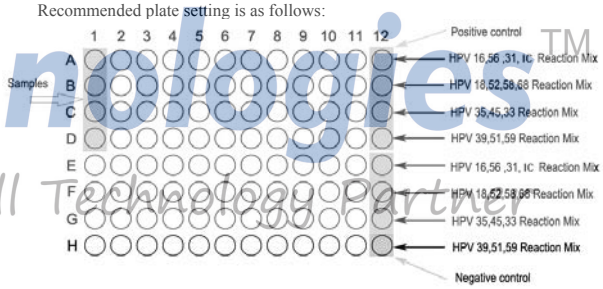
9.3 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
2) Pipet **36µl** Master Mix with micropipets of sterile filter tips to each *Real time* PCR reaction plate/tubes. Separately add **4µl** DNA sample template, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.

Recommended plate setting is as follows:



3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

94°C for 2min	1cycle
93°C for 10sec, 62°C for 31sec (Fluorescence measured at 62°C)	40cycles

Selection of fluorescence channels	
FAM	Target Nucleic Acid
HEX/VIC/JOE	Target Nucleic Acid
TEXAS RED	Target Nucleic Acid
Cy5	Target Nucleic Acid

5) ⚠ If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher.**
6) ⚠ If you use LightCycler®480 system, please do **color compensation** before detection.

10. Baseline setting: just above the maximum level of molecular grade water.

11.Quality control:

Controls	Master Mix	Channel	FAM	HEX	TEXAS RED	Cy5
Molecular Grade Water (Negative control)	1	16,56,31 ,IC	UNDET	UNDET	UNDET	
	2	18,52,58,68	UNDET	UNDET	UNDET	UNDET
	3	35,45,33	UNDET	UNDET	UNDET	
	4	39,51,59	UNDET	UNDET	UNDET	
Positive Control	1	16,56,31,IC	Ct≤35	Ct≤35	Ct≤35	
	2	18,52,58,68	Ct≤35	Ct≤35	Ct≤35	Ct≤35
	3	35,45,33	Ct≤35	Ct≤35	Ct≤35	
	4	39,51,59	Ct≤35	Ct≤35	Ct≤35	

12. Data Analysis and Interpretation

1)The Ct value shows ≤35. **The result is positive: The sample contains some serotype of HPV DNA.** The following results are possible:

Master Mix	Channel	FAM	HEX/VIC/JOE	TEXAS RED	Cy5
1	16,56,31,IC	HPV16	HPV56	HPV31	
2	18,52,58,68	HPV18	HPV52	HPV58	HPV68
3	35,45,33	HPV35	HPV45	HPV33	
4	39,51,59	HPV39	HPV51	HPV59	

2) The Ct value shows 35~40, please repeat again. **If the result still shows 35~40, it can be considered negative.** But the clinical samples in channel of Cy5 in 16,56,31,IC Reaction Mix should be positive. Otherwise, the negative result of the sample is invalid. Please refer to section 9.2 in detail.
3) In any channel no signal is detected in any one of HPV Master Mix. **The sample does not contain any one of 13 Serotypes HPV. It can be considered negative.**