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CAUTION! Professional use only.
Follow instruction manual before use.
Reading and carefully following these warnings will minimize usage errors, other possible risks and hazards.
Wear safety glasses, clothes, and gloves.

HPV16/HPV18 Qualitative RT-qPCR Detection Kit

User Guide

Catalog Number	Pack Size
ZT-HPV1618-01-100	100 Tests
ZT-HPV1618-01-500	500 Tests
ZT-HPV1618-01-1000	1000 Tests

Content and Storage Condition

Content	Quantity (20µL/Reaction)			Shipping	Routine Storage
	100 Tests	500 Tests	1000 Tests		
16/18 RT-qPCR Mix	1,4 mL	5 x 1,4 mL	10 x 1,4 mL	-20°C	-20°C
Positive Control	0.05 mL	0.05 mL	0.1 mL	-20°C	-20°C
Negative Control	0.05 mL	0.05 mL	0.1 mL	-20°C	-20°C

Features

- Kit content must be stored at -20°C until expiry date.
- Freeze thaw cycles for 16/18 RT-qPCR Mix is up to 5 times.
- To avoid repeated freezing and thawing as well as to minimize the contamination risk of stock solutions of reagents, it is highly recommended to divide large-volume stocks into several smaller aliquots and store them at -20°C.
- Kit is compatible with both qPCR cyclers that do not require and require an internal reference dye for normalization of fluorescent signal.

Product Description

Human papillomavirus (HPV) pertains to the genus of papilloma viruses within the family Papovaviridae. Currently, more than 100 HPV types have been identified, approximately 30 of which possess the capability to infect the urogenital tract epithelium in humans.

DNA diagnostics for HPV infection are presently considered fundamental for cervical cancer screening and prevention.

The presence of HPV can be utilized to modify the examination plan and the frequency of patient monitoring. A positive test result holds significant diagnostic value, especially for women over the age of 30.

HPV16/HPV18 QUALITATIVE RT-qPCR DETECTION KIT is in vitro DNA test, which is intended for the specific identification of human papillomavirus 16,18 in human biological samples.

HPV16/HPV18 QUALITATIVE RT-qPCR DETECTION KIT comes in 3 different tubes and contains all the components necessary to perform RT-qPCR in a single tube on qPCR cyclers.

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HPV16/HPV18 QUALITATIVE RT-qPCR DETECTION KIT Protocol

Clean the workbench to be worked with 0.5-1% (w/v) sodium hypochlorite first, then with 70% Ethyl alcohol. Thaw the reagents at 4°C on a cold shelf or on ice. If your time for work is limited, thaw them at room temperature. Mix each reagent several times by gently pipetting up and down, then centrifuge briefly. To avoid many freeze-thaw processes, please divide the mixes into sterile dnase/rnase free tubes at the first use. **Prevent the fluorescent marked probes in the 16/18 RT-qPCR Mix from being exposed to light and perform the operation in a dark environment every time you use it.** Determine the total number of reactions per assay run. Each assay run should include the following:

İ) One Positive Control Sample that uses the PC (Positive Control) provided in the kit, as template.
İİ) One Negative Control Sample that uses the NC (Negative Control) provided in the kit, as template.
İİİ) Total the number of Collected Samples

Component	Volume Per 20 µl Reaction		
	Positive Control	Negative Control	Test Sample
16/18 RT-qPCR Mix	14 µL	14 µL	14 µL
Test Sample	-	-	6 µL
PC	6 µL	-	-
NC	-	6 µL	-

Run your RT-qPCR assay as shown in table below. NOTE: When programming your RT-qPCR run, choose the detection channel(s) as FAM, HEX and ROX.

Steps		Temperature	Time
Reverse transcription		50°C	15 min
Enzyme activation		95°C	30 sec
X 45 Cycles	Denaturation	95°C	10 sec
	Annealing/Extension	60°C (READ)	30 sec

Collect and analyze the data according to the instrument- specific instructions. Verify the amplification curve.

Target	Fluorophore/Channel
HPV16 target gene	FAM
HPV18 target gene	ROX
Internal gene	HEX

Data Analyses

	FAM	HEX	ROX	Interpretation
PC	Ct < 38	Ct < 38	Ct < 38	HPV16 and HPV18 Positive
NC	-	Ct < 38	-	Negative
Test Samples	Ct < 38	Ct < 38	Ct < 38	HPV16 and HPV18 Positive
	Ct < 38	Ct < 38	-	HPV16 Positive, HPV18 Negative
	-	Ct < 38	Ct < 38	HPV18 Positive, HPV16 Negative
	-	-	-	Invalid Test
	-	Ct < 38	-	Negative

Safety and Hazards

General Safety

Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

General Requirements for Good Practices on PCR and RT-QPCR

Laboratory Setup

To prevent contamination of the reaction mixture by previously amplified target sequences, it shall be ensured that separate work areas with their own apparatus are available. If possible, maintain separate work areas, dedicated equipment, and supplies for: Sample preparation, PCR setup, PCR amplification, Analysis of PCR products.

Personnel

HPV16/HPV18 QUALITATIVE RT-qPCR DETECTION KIT is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the molecular techniques and in vitro diagnostic procedures. Different sets of laboratory coats should be worn pre- and post-PCR. Disposable gloves should be worn at sample preparation and when setting up RT-qPCR.

Protection of Product Performance and Analysis Efficiency

The components in the kit should not be mixed with components with different lot numbers or chemicals of the same name but from different manufacturers. Master stock reagents should be kept on the cold block during the PCR setup: if possible, the PCR setup should be performed on the cold block. Kit components should be mixed by gently shaking before use.

Preventing Contamination

The kit should be stored away from nucleic acid sources and qPCR amplicons. The micropipettes used for pipetting qPCR mixes and template nucleic acids should be separate. Filtered and nuclease-free pipette tips should be used.

Plate Layout Suggestions

In multi-targeted PCR runs, separate different targets by a row or by a column if enough space is available. If possible, put at least one well between unknown samples and controls.

Warning

Do not preserve the product when the package is damaged.

Assay Limitations

- HPV16/HPV18 QUALITATIVE RT-qPCR DETECTION KIT is intended for use in a laboratory environment by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- A false negative result may occur if a specimen is improperly collected, transported, or handled.
- Detection of viral RNA may be affected by patient factors and/or stage of infection.

Troubleshooting

Observation	Possible Cause	Recommended Action
Failure to detect FAM/HEX-/ROX signals in Positive Control wells or HEX signals in Negative Control wells	PCR amplification failure.	Make sure that the mix and PC/NC samples are pipetted correctly. Check that the thermal cycler settings and amplification program are correct. If there are no errors in them, renew the reagents and repeat the reaction. Contact with the manufacturer.
In the Negative Control wells, target-specific signals (FAM and/or ROX) are detected.	Contamination of the PCR	The cause of contamination may be due to hand errors made in sample processing, reagent contamination or environmental factors. Decontaminate the benchtop surfaces and other equipment where the PCR process is performed with 70% Ethyl alcohol and repeat the PCR process. Pipette Positive Control reactions last to avoid cross-contamination. Be sure to pay attention to the points in the section "General Requirements and Warnings on PCR and RT-qPCR Good Practices".
Detection of FAM and/or ROX signal in test sample wells, while HEX signal is not detected	A high copy number of target nucleic acid exists in samples, resulting in preferential amplification of the target-specific NA.	Repeat the PCR process for this sample. If the problem persists, dilute the sample with sterile nuclease-free water in a 1:1 ratio and repeat the PCR process. If the problem persists, dilute the sample with sterile nuclease-free water in a 1:10 ratio and repeat the PCR process. If all the results are the same, consider positive of the patient sample.
No signal is detected in any channel in the test sample wells	Inhibition Problem	Dilute the test sample in a ratio of 1:10 and repeat the PCR procedure. If the diluted sample does not show a positive result in the HEX channel, request a new sample from the patient. If the problem persists, contact with the manufacturer.