Pioneer Research Anahita Company
No.157
Danesh street
Technology Park Pardis
Tehran/ Iran

DATA SHEET
Versions: 01
Revision date: 25/11/2023

1. Identification

Product name: HPV Genotyping Real-Time PCR Kit

Reactions: 12 rxns

Cat. No.: MB287868

2. Description

Human papillomavirus (HPV), double-stranded DNA viruses without They are membranes that replicate in the nucleus of epithelial cells and cause progressive lesions. There are many of these viruses in the world It is common and transmitted through sexual contact. Most HPV infections. They have a benign clinical outcome and disappear by themselves. More than 150 types There are HPVs that are divided into two high-risk categories based on their carcinogenic potential. HPV and low risk are classified. At least 14 types of HPV as High-risk types are known to cause cervical cancer in carrier women They will be. Approximately 70% of invasive cervical cancer cases worldwide They are caused by HPV16 and HPV18 infection. Infection with types 16 and 18 Comparison with other high-risk genotypes with a higher risk of disease progression Accompanied. Identification of high-risk genotypes in the management of cervical cancer as a predictor and as a second test in doubtful cases after Pap smear tests are very valuable. HPV Genotyping Kit for detection and genotyping of 14 high-risk types of HPV And also 68, 66, 59, 58, 56, 52, 51, 45, 39, 35, 33, 31, 18, 16 included Two low-risk types 6 and 11 have been designed. Primer and probes designed in This kit targets L2, L1 and E2 areas. To detect HPV type After extracting DNA from the samples of human papilloma viruses in Cervical cells collected in liquid cytology environment, method TaqMan Real-Time PCR is performed. Internal control gene of human beta globin in this kit in addition to guaranteeing the extraction quality of collected samples It prevents false negative reports. This kit can be used in Real-Time PCR devices with the ability to read in four green channels (yellow FAM). orange (ROX) and red (CY5).

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3. Kit Contents

Component	Quantity
CAPITAL qPCR Mix	260 µl
Primer/Probe A	65 µl
Primer/Probe B	65 µl
Primer/Probe C	65 µl
Primer/Probe D	65 µl
negative control	100 µl
positive control	100 µl

4. Storage specifications

The kit should be kept at a temperature of -20 to -25 degrees Celsius. From melting and freezing

The repetition of the kit should be avoided as much as possible

5. Applications

All molecular biology applications, such as:

6. Assay Procedure

method

Take the contents of the kit out of the packaging and let it cool at room temperature to melt the preparation space of Mr. Mix should be from the space of adding samples be separate in order to prevent contamination of the samples, the positive control kit in the space Preparation Do not open Mr. Mix. For genotyping each sample should 4 Prepare a real-time microtube. According to the number of tested samples Add positive control and negative control to the determined amount of the following mixtures do it.

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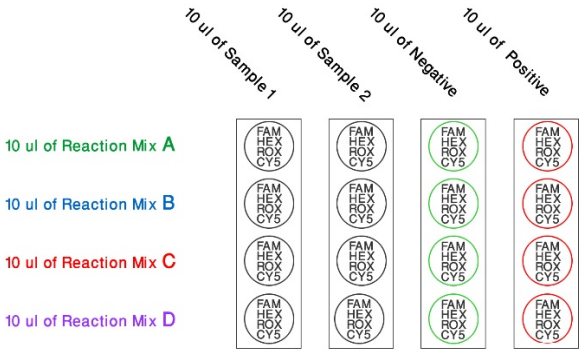
Reaction Mix **A**: 5ul of Primer/Probe A + 5ul of 4x Capital qPCR Mix
Reaction Mix **B**: 5ul of Primer/Probe B + 5ul of 4x Capital qPCR Mix
Reaction Mix **C**: 5ul of Primer/Probe C + 5ul of 4x Capital qPCR Mix
Reaction Mix **D**: 5ul of Primer/Probe D + 5ul of 4x Capital qPCR Mix

- The amount of 10 microliters of each of the four prepared master mixes C, B, A and Add D to the respective microtubes and then to each of Microtubes containing master mixes C, B, A and amount of 10 microliters Add sample DNA until the final volume reaches 20 microliters.
- 10 microliters of negative control to each of the four microtubes Add C, B, A and D master mixes.
- 10 microliters of positive control to each of the four microtubes Add C, B, A and D master mixes



After closing the lid of the microtubes, put them inside the real time machine. For each sample, select green (FAM), yellow (HEX), orange (ROX) and red (CY5) channels. In devices such as ABI 7500 and Gentler, the reference dye. Set None too Passive Reference Dye.

Cycle	Temperature	Time	Step
Activation	3 min	95°C	1
45	Denaturation	10 sec	95°C
62°C Reading on **FAM (Green)** **HEX (Yellow)** **ROX (Orange)** **CY5 (Red)**	Annealing	30 sec	
Cooling	25 sec	35	1

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Analyze

After the end of the reaction, the multiplication curves in all four channels are as follows are examined separately.

1. In each negative control test in ROX, HEX, FAM and CY5 channels Mixes C, B, A and Mix D should not have Ct less than 40. Ct four male channels In positive control, it should be between 24 and 26 in all four master mixes.
2. Then the internal control multiplication curve (Mix A: CY5) should be analyzed. If the curve If it is sigmoid and Ct is less than 35, the test is reliable.
3. If Ct sigmoid curve in HEX, FAM and ROX channels in Mix A is smaller and or be equal to 38, sample for HPV18, HPV45 and HPV16 respectively it's positive.
4. If sigmoid curve Ct in ROX, HEX, FAM and CY5 channels in Mix B be less than or equal to 38, samples for HPV68 and HPV35 respectively. It is positive for HPV51 and HPV59
5. If Ct sigmoid curve in ROX, HEX, FAM and CY5 channels in Mix C be less than or equal to 38, samples for HPV52 and HPV58 respectively. It is positive for HPV56 and HPV66
6. If sigmoid curve Ct in ROX, HEX, FAM and CY5 channels in Mix D be smaller or equal to 38, samples for HPV31 and HPV39 respectively. It is positive for HPV11 or HPV6 and HPV33

	FAM Green	HEX Yellow	ROX Orange	CY5 Red	Positive for
Mix A	Ct ≤ 38	-	-	-	HPV 45
	-	Ct ≤ 38	-	-	HPV 18
	-	-	Ct ≤ 38	-	HPV 16
	-	-	-	Ct ≤ 35	β -Globin
Mix B	Ct ≤ 38	-	-	-	HPV 35
	-	Ct ≤ 38	-	-	HPV 68
	-	-	Ct ≤ 38	-	HPV 59
	-	-	-	Ct ≤ 38	HPV 51
Mix C	Ct ≤ 38	-	-	-	HPV 58
	-	Ct ≤ 38	-	-	HPV 52
	-	-	Ct ≤ 38	-	HPV 66
	-	-	-	Ct ≤ 38	HPV 56
Mix D	Ct ≤ 38	-	-	-	HPV 39
	-	Ct ≤ 38	-	-	HPV 31
	-	-	Ct ≤ 38	-	HPV 33
	-	-	-	Ct ≤ 38	HPV 6 or 11

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7. If the internal control Ct is higher than 35, extract DNA again

Analytical sensitivity of the kit

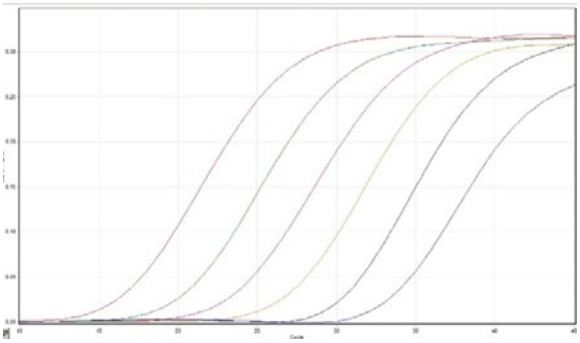
The analytical sensitivity of the kit was investigated using the positive control of the kit and 66, 59, 58, 56, 52, 51, 45, 39, 35, 33, 31, 18 and 16 for types 68 and also two low-risk types 6 and 11, equivalent to 500 copies per milliliter of the sample.

Specificity of the analytical kit

Investigating cross-reactivity using in silico analysis NCBI Primer Blast and in Database nr showed that the primer/probe kit with bacteria (Taxid: 2), mushrooms (Taxid: 4751) and humans (Taxid: 9606) cross reaction
does not give in the in-silico study of viruses (Taxid: 10239) only papilloma virus and 66, 59, 58, 56, 52, 51, 45, 39, 35, 33, 31, 18, 16 human types 68 and also 2 low-risk types 6 and 11 multiply.

Clinical evaluation of the kit

In order to clinically evaluate the performance of 14 High-Risk HPV Genotyping Kits in total 160 samples including 60 HPV positive samples and 100 negative samples were performed and with the kit. Compared HPV Genotypes 14 Real-TM Quant (Sacace)The sensitivity of the kit for each of the 14 high-risk genotypes and 2 low-risk genotypes is above 95The percentage and specificity were estimated to be above 98%



1 to 10 dilution of HPV 45 positive sample in FAM channel from M

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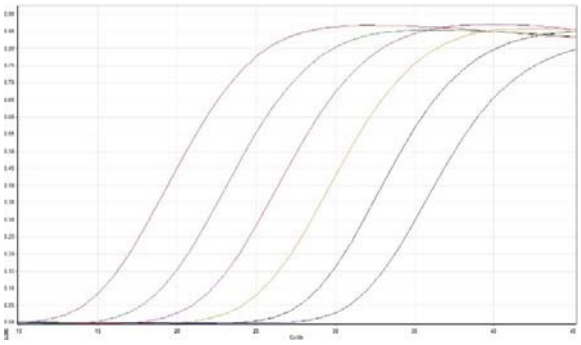
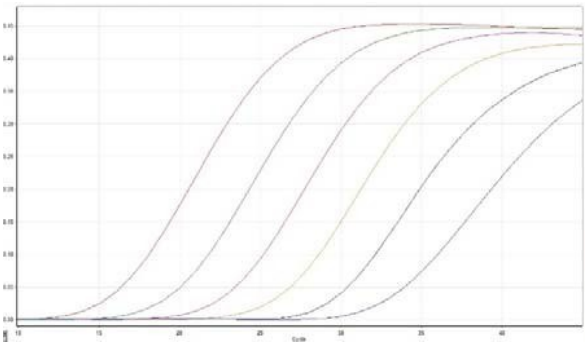


1 in 10 dilution of HPV 18 positive sample in HEX channel from Mix A



1 to 10 dilution of HPV 16 positive sample in ROX channel from Mix A

7. Safety

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.

8. Quality Certifications

NONE

9. Further information

All products have been manufactured by Tose zist fanavar Mellat Company in the Iran

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10. Other Kits

monoclonal antibodies

Anti-CD3
Anti-CD9
Anti-CD9A
Anti-CD34
Anti-CD44
Anti-CD63A
Anti-CD69
Anti-CDX2
Anti-Her2
Anti-Oct4
Anti-Nanog
Anti-Nestin antibody
Anti-Toxoplasma gondii
Anti-Vimentin
Anti-c-Kit
Anti-Ferritin
Anti-Ki67

Mouse antibody
Anti-beta Actin
Mouse IgG1 (Isotype Control)
Mouse IgG (Isotype Control)

polyclonal antibodies

Anti-Human IgA
Anti-Human IgD
Anti-Human IgE
Anti-Human IgG
Anti-Nanog
Sheep IgG
Sheep anti-Mouse Ig
Goat Anti-Human IgG
Goat Anti-Human IgM
Goat Anti-Rabbit IgG
Goat Anti-Mouse IgG
Mouse IgG2a
Anti-beta Actin
anti-CD133
Rabbit IgG (Isotype Control)
anti-CD133 (FITC)

Second antibodies

Sheep Anti-Rabbit Ig
Sheep Anti-Mouse IgG
Goat Anti-Rabbit IgG
Goat Anti-Mouse IgG
Streptavidin
Anti-beta Actin antibody
Rabbit Anti-Sheep Ig Antibody

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Rabbit Anti-Mouse IgG2a
Rabbit Anti-Mouse IgG2b
Rabbit Anti-Mouse IgG3
Rabbit Anti-Human IgG
Rabbit Anti-Mouse IgG
Goat Anti-Human IgG
Sheep Anti-Rabbit IgG (FITC)
Sheep Anti-Mouse IgG (FITC)
Goat Anti-Rabbit IgG (FITC)
Goat Anti-Human IgG (FITC)
Goat Anti-Mouse IgG (FITC)
Goat Anti-Mouse IgG (PE)