Viga Genotyping HPV Molecular Diagnostic Kit

Store at-20 to -25˚C

In darkness

25 rxn

Cat NO: MD003060

100 rxn

Cat NO: MD003061

By ROJE

Edition, 07/2023

Kit Content

Kit content	25 Preps	100 Preps
Q-ROMAX, 4X	600µl	2400µl
Pro1 HPV Mix	350µl	1400µl
Pro2 HPV Mix	350µl	1400µl
Pro3 HPV Mix	350µl	1400µl
Pro4 HPV Mix	350µl	1400µl
Positive Control	100µl	600µl
Water (PCR Grade)	150µl	600µl

Recommended Starting Material

Before starting any tests, each component must be melted at room temperature, then homogenize gently and spin. Avoid repeated freeze-thaw cycles.

Buffer Preparation

Take out each component from the kit and place them on the benchtop. Allow the reagents to equilibrate to room temperature, then homogenize gently and spin. Follow table 1 to prepare components; the isolated sample volume is 5µl. Follow table 2 for Real time-PCR run.

Table 1: Preparation of components per single reaction

components	Volume
Q-ROMAX, 4X	6µl
Pro1 HPV or Pro2 HPV or Pro3 HPV or Pro4 HPV Mix	14µl
Isolated DNA	5µl

Thermal Profile

Table 2: Thermal profile for Viga Genotyping HPV Molecular Diagnostic Kit.

Stage	Temperature	Incubation Time	Cycle Numbers
Pre-Denaturation	95 °C	1 min	1
Denaturation	95 °C	10sec	45
Annealing and acquisition on channel Green, Yellow, Orange	57°C	30sec	

Protocols

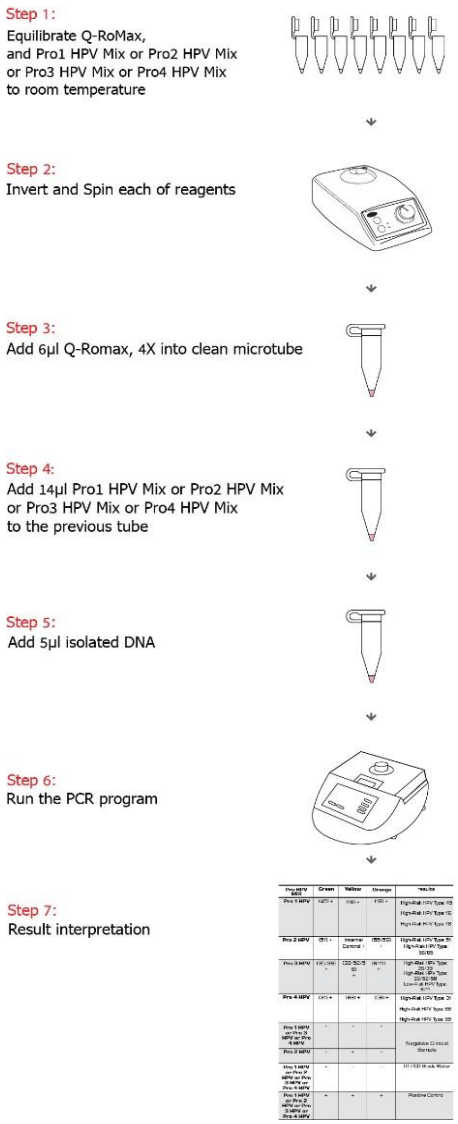

Figure 1: preparation of reagents, PCR run, and interpretation of results.

Result interpretation

Data analysis for each gene should be performed separately using a manual threshold.

Use the following table for results interpretation, showing that Pro HPV1 to Pro HPV4 mixes are detectable in identified channels.

ProMIX	Green	Yellow	Orange
Pro1 HPV	45	16	18
Pro2 HPV	51	Internal Control	56/66
Pro3 HPV	35/39	33/52/58	6/11
Pro4 HPV	31	68	59

A negative control is used as contamination control. The magnitude increase of the

Fluorescence curve in the negative control does not cross the threshold. If Ct is less than 35 (Ct<35), it is considered as possible contamination. Strong signals above 35 in the NTC can be PCR artifacts, which in these cases, the shape of the curve can be considered (the S-shaped curve is typical for a positive result).

Internal control should be positive for all clinical specimens at Ct 35 or less than 35, indicating sufficient nucleic acid from the human gene and the sample has acceptable quality.

Internal control curve with Ct>37 or without Ct indicates low sample concentration or inhibitors in the reaction (the isolated sample is recommended to dilute at least ½). If the test result is not acceptable again during the retest, another new sample should be taken from the patient, and the test must be repeated.

A positive clinical specimen should have Ct≤37 for genes or have two positive genes.

If the expected positive reaction is not achieved (typical S-shaped curve), the performed test is not acceptable. The test must be repeated based on kit instructions accessible in the kit catalog.

Determine the reason for the failure of positive control, take the corrective action, and document corrective action results.

To determine the result of samples with CT>35, pay attention to the clinical symptoms and history of the patient.

Table 3: Control conditions for a valid PCR Run

Pro HPV Mix	Green	Yellow	Orange	results
Pro 1 HPV	(45) +	(16)+	(18) +	High-Risk HPV Type: 45 High-Risk HPV Type: 16 High-Risk HPV Type: 18
Pro 2 HPV	(51) +	Internal Control +	(55/56) +	High-Risk HPV Type: 51 High-Risk HPV Type: 56/65
Pro 3 HPV	(35/39) +	(33/52/ 58)+	(6/ 11)+	High-Risk HPV Type: 35/39 High-Risk HPV Type: 33/52/58 Low-Risk HPV Type: 6/11
Pro 4 HPV	(31) +	(68) +	(59) +	High-Risk HPV Type: 31 High-Risk HPV Type: 68 High-Risk HPV Type: 59
Pro 1 HPV or Pro 3 HPV or Pro 4 HPV	-	-	-	Negative Clinical Sample
Pro 2 HPV	-	+	-	
Pro 1 HPV or Pro 2 HPV or Pro 3 HPV or Pro 4 HPV	-	-	-	RT-PCR Grade Water
Pro 1 HPV or Pro 2 HPV or Pro 3 HPV or Pro 4 HPV	+	+	+	Positive Control