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

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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	150µl	600µl
Water (PCR Grade)	150µl	600µl

Recommended Starting Material

Before starting any tests, each component must be melted, vortexed, and centrifuged briefly. Avoid repeated freeze-thaw cycles.

Before Start

Take out each component from the kit and place them on the benchtop. Allow the reagents to equilibrate to room temperature, then briefly vortex each tube for later use.

Buffer Preparation

Take out each component from the kit and allow the reagents to equilibrate to room temperature. Before use, vortex components briefly. The whole volume of isolated nucleic acid should be 5µl. Follow table 1 to prepare buffers and table 2 for 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	3 min	1
Denaturation	95 °C	10sec	45
Annealing and acquisition on channel Green and Yellow	57°C	30sec	

Protocols

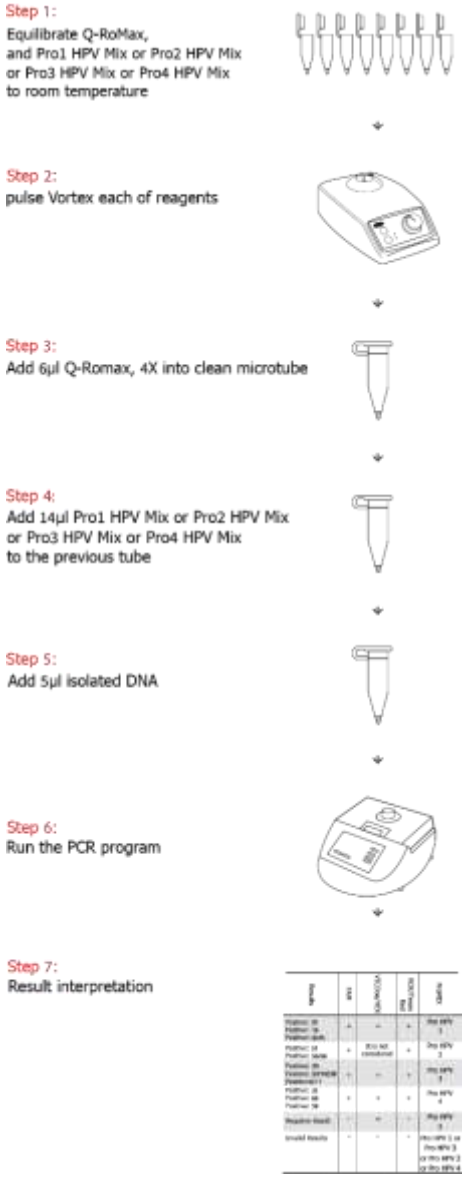

Figure 1: preparation of reagents, PCR run, and interpretation of results.

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	31	16	18/45
Pro2 HPV	51	Internal Control	56/66
Pro3 HPV	39	33/52/58	6/11
Pro4 HPV	35	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≤40 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.

For more information about positive and negative specimens, refer to table 3.

Table 3: Control conditions for a valid PCR Run

results	FAM	Yakima Yellow	ROX/Texas Red	ProMIX
Positive:31 Positive: 16 Positive:18/45	+	+	+	**Pro HPV 1**
Positive:51 Positive:56/65	+	It is not considered	+	**Pro HPV 2**
Positive:39 Positive:33/52/58 Positive:6/11	+	+	+	**Pro HPV 3**
Positive:35 Positive:68 Positive:59	+	+	+	**Pro HPV 4**
Negative Result	-	+	-	**Pro HPV 2**
Invalid results	-	-	-	**Pro HPV 1 or Pro HPV 2 or Pro HPV 3**
				or Pro HPV 4