For in vitro diagnostic use only.
For professional use only.

Cat No: BS-GHPV-S-25/BS-GHPV-S-100

Human Papillomavirus Genotyping qPCR Panel

Package Insert

Table 1. Kit Content

Component		Intended Use	48 Reactions	96 Reactions
GHPV Rxn 	**A:** GHPV Oligo Mix 1	Specific nucleic acid amplification and detection: **FAM**: Human papillomavirus 16 **HEX:** *Human* (IC-Internal Control) **ROX:** Human papillomavirus 39 **CY5:** Human papillomavirus 31	48 GHPV Rxn strips	96 GHPV Rxn Strips
	B: GHPV Oligo Mix 2	**FAM** Human papillomavirus 33 **HEX:** Human papillomavirus 35 **ROX:** Human papillomavirus 18		
	C: GHPV Oligo Mix 3	**FAM:** Human papillomavirus 51 **HEX:** Human papillomavirus 52 **ROX** Human papillomavirus 56 **CY5:** Human papillomavirus 66		
	D: GHPV Oligo Mix 4	**FAM:** Human papillomavirus 59 **HEX:** Human papillomavirus 45 **ROX:** Human papillomavirus 68 **CY5:** Human papillomavirus 58		
	E: GHPV Oligo Mix 5	**FAM:** Human papillomavirus 40 **HEX:** Human papillomavirus 70 **ROX:** Human papillomavirus 82 **CY5:** Human papillomavirus 44		
	F: GHPV Oligo Mix 6	**FAM:** Human papillomavirus 53 **HEX:** *Human* (IC-Internal Control) **ROX:** Human papillomavirus 42 **CY5:** Human papillomavirus 73		
	G: GHPV Oligo Mix 7	**FAM:** Human papillomavirus 69 **HEX:** Human papillomavirus 61 **ROX:** Human papillomavirus 11 **CY5:** Human papillomavirus 54		
	H: GHPV Oligo Mix 8	**FAM:** Human papillomavirus 6 **HEX:** Human papillomavirus 43 **ROX:** Human papillomavirus 26		
2X qPCR Mix		Optimized ready-to-use mix for qPCR assay	48 x 90 µL	96 x 90 µL
NTC		Negative Control	1 x 1000 µL	
PC-GHPV 1 / PC-GHPV 2 / PC-GHPV 3 / PC-GHPV 4 / PC-GHPV 5 / PC-GHPV 6 / PC-GHPV 7 / PC-GHPV 8		Positive Control (PC)	1 x 100 µL	

Table 2. Transport Condition, Storage Condition, and Shelf Life of the Components

Component	Transport Condition	Storage Condition*	Shelf Life
2X qPCR Mix	(-22) – (+8) °C	(-22) – (-18) °C	12 Months
Oligo Mix		(-22) – (-18) °C	
PC		(+2) – (+8) °C	
NTC		(+2) – (+8) °C	

*Each reagent stored at storage temperature can be used until the expiration date indicated on the tube following the first opening. The kit's expiration date is determined by the expiration date of the reagents.

Table 3. Components Required but Not Included with The Test

Components Required but Not Included with The Test
1. CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad) Real-Time PCR systems
2. Micropipettes and filtered pipette tips (nuclease-free) suitable for transferring 1-10, 10-100, and 100-1000 µL of liquid
3. A centrifuge or Mini-spin
4. Vortex
5. Reaction tubes and caps/films specific to qPCR instruments and compatible with reaction volume

Table 4. Intended Use, Test Principle, and Analytical Specifications

Function	Aid to diagnosis	**Sample Type(s)**	Table 5
Analyte(s)	Table 1	**Nucleic Acid Preparation Method(s)**	Table 5
Qualitative/Quantitative	Qualitative	**Validated PCR Instrument(s)**	Table 3
Test Principle	Real-Time PCR (qPCR)	**Results Interpretation and Reporting**	Automated (Sigmoida software)
Automated/Manual	Manual	**Inclusivity and Exclusivity**	Validated on the reference strains and the field isolates
Intended Users	Professional use	**Limit of Detection (LoD)**	Table 5
Target Population	Individuals with the suspected infection	**Sensitivity and Specificity**	99.50% and 98.48%

Table 5. Collection, Storage, and Transfer of Clinical Specimens / Nucleic Acid Preparation Methods and the Respected LoD Values

Sample Type**	Sample Transfer	Sample Storage	Nucleic Acid Preparation Method	LoD (IU/mL)
Cervical, vaginal, and penile swab samples	***vNAT® Transfer Tube*** (Cat. No: BS-NA-513m/BS-NA-513)	3 months at (+2) – (+8) °C 1 year at -20 °C	Nucleic acid preparation is not required.	2000-4800
	Viral Transport Medium (VTM) (CDC SOP#: DSR-052-05)	3 days at (+2) – (+8) °C 1 year at -70 °C	***RINA™ M14 Nucleic Acid Extraction Device*** (Robot Catalog No: RINA-M14-01, Kit Cat. No: RN-NA-101) ***Zybio EXM3000 Nucleic Acid Isolation System*** (Robot Model No: EXM3000, Kit Cat. No: ZFNAE01)	500-1200
	Sterile tube/container that does not contain any preservatives			

**Clinical specimens should be collected by a healthcare provider in accordance with national/international clinical specimen collection regulations.

1. qPCR Application Protocol

Program the qPCR device as follows, add the reagents into the qPCR tubes, close the tubes, place them into the qPCR instrument, and start the run. (Table 6 and Table 7)

Table 6. Reaction Setup

Reaction Type	Strip to be used	The sample is added to one 2X qPCR Mix tube in the amounts given below, mixed with pipetting, and the mix is distributed into the strip wells in the amount given in the right column.		The amount to be distributed to A, B, C, D, E, F, G and H wells
Patient Sample	**GHPV** 1 strip per patient	2X qPCR Mix	90 µL	**15 µL**
		Nucleic acid extract obtained from a patient sample	45 µL	
		Total	**135 µL**	
Negative Control (NTC)	**GHPV** 1 strip per run	2X qPCR Mix	90 µL	**15 µL**
		NTC	45 µL	
		Total	**135 µL**	
Positive Control (8 different PC tubes)	**GHPV** 1 strip per run	2X qPCR Mix	90 µL	**15 µL**
		PC Mix	45 µL	
		Total	**135 µL**	

Table 7. qPCR Program Details

Step	Cycle No.	Temperature	Duration
Enzyme Activation	1 Cycle	52 °C	3 min
Pre-Incubation	1 Cycle	95 °C	10 sec
Denaturation	12 Touchdown Cycles: 1 °C decrement in annealing temperature per cycle	95 °C	1 sec
Annealing and Extension		67 °C – 56 °C	15 sec
Denaturation	35 Cycles	95 °C	1 sec
Annealing and Extension		55 °C	15 sec
Detection (Reading)		FAM/HEX/ROX/CY5	

WARNING: qPCR thermal program and plate setup file should be downloaded from the QR code on the left or the link below
https://www.bioeksen.com.tr/files/BS_TD_3RT153555

2. Interpretation of the Assay Results Using The "Sigmoida" Software

The data produced by the instruments must be evaluated and reported using the Sigmoida software. The result files opened with the "Sigmoida" software will be analyzed automatically. Below are examples of results that can be achieved with the Sigmoida software:

Negative: The sample tested is negative for the tested agent.
Positive: The sample tested is positive for the tested agent.
Contamination: Repeat the analysis paying attention to the "Warnings and Limitations" section.
Invalid: Sampling isn't successfully done, or there is a problem during the sample transportation. A new sample from the same patient should be collected and tested again.
Reagent Problem: Test the PCs provided with the kit setting up the PC reactions, as shown in Table 6. If the test result is positive, the run is valid. In case the software generates a "Reagent Problem" again, contact the manufacturer.

The Sigmoida Software Algorithm

- The Sigmoida software sets the threshold level to **750 RFU** for **CFX96 Touch™ /CFX96™ Dx/CFX Opus 96™ /CFX Opus 96™ Dx (Bio-Rad)** Real-Time PCR systems to calculate Cq values and does not change any other analysis options.
- All sigmoidal curves above the threshold level are recorded as "**positive.**" Non-sigmoidal curves are recorded as "**negative.**"

In case the result is positive, it is reported as follows:

- If *25≤Cq≤35 → "Low Positive"*
- *If 16≤Cq<25 → "Positive"*
- *If Cq<16 → "High Positive"*

Table 8. Expected Performance of Kit Controls

Control Type	Control Name	Purpose	Expected Results and Cq Values	
			IC (HEX)	Target
Negative Control	NTC	Contamination control during qPCR	**Not detected** (No Cq)	**Not detected** (No Cq)
Positive Control	PC	Reagent integrity	**Detected** (Cq≤33)	**Detected** (Cq≤33)
Internal/Extraction Control	IC	To monitor the integrity of nucleic acid extraction and qPCR from each sample	**Detected** (Cq≤33) If the IC Cq>33, check the target Cq.	If the target has a valid Cq value according to the result interpretation criteria, IC is valid.

If any control does not work as described above, the run is reported as follows:

1. **Contamination:** If Cq≤35 in any NTC test channel.
2. **Reagent Problem**: In case a sigmoidal curve with a Cq≤33 cannot be obtained for any of all the samples tested in the run, including the controls.
3. **Invalid:** If the sample has a Cq>33 in the HEX channel of the test tube and no Cq in the other channels.

If all the controls are valid, the results are interpreted as follows:

Table 9. Interpretation of Patient Results

Target	IC	Result Interpretation	Action
Positive (+)	**Positive (+)** or **Negative (-)**	Results are valid, Target is detected.	It is reported as positive.
Negative (-)	**Positive (+)**	Results are valid, Target is not detected.	It is reported as negative.

3. Warnings and Limitations

1. False-negative results may occur if inadequate numbers (lower than the LoD) of organisms are present in the specimen.
2. Mutations within the target regions could affect primer and/or probe binding, resulting in failure to detect the presence of agents.
3. A false-negative result may occur if a specimen is improperly collected, transported, or handled.
4. The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
5. Test procedures should be performed by personnel trained in the use of the kit.
6. Sample tubes should always be kept closed except for liquid transfers.
7. Filtered and nuclease-free pipette tips should be used for sample transfer.
8. The components in the kit should not be used together with different lot numbers or chemicals of the same name but from different manufacturers.
9. ***The caps of the reaction tubes must not be opened after the PCR run.*** The PCR tubes should be placed in a bag and thrown away after the bag is tightly closed.
10. The surfaces of the workbenches should be wiped with freshly diluted 10% bleach (0.5% NaClO) at the beginning and end of each day.
11. Disposal of waste must be carried out in accordance with local, state, and federal regulations.
12. The use of cotton or calcium alginate swabs or swabs with wooden sticks can lead to false negative results since they may contain substances that inactivate some viruses and inhibit PCR.

4. Explanation of Symbol

Symbol	Title of Symbol	Symbol	Title of Symbol	Symbol	Title of Symbol
CE	European Conformity CE Mark	LOT	Batch code		Keep away from sunlight
IVD	*In vitro* diagnostic *medical device*	REF	Catalogue number		Protect from heat and radioactive sources
	Manufacturer	NON STERILE	Non-*sterile*		Do not use if package is damaged and consult *instructions for use*
	Use-by date		Consult *instructions for use* or consult electronic *instructions for use*		Keep dry
CONTROL -	Negative control		Caution		Keep upright
CONTROL +	Positive control		Temperature limit	Σ	Contains sufficient for *<n>* tests
CONTROL	Control				

5. Manufacturer and Technical Support

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Notice to User: Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr**" within 24 hours.

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