VisionArray
HPV Primer Kit 2.0

REF VP-0001-50 Σ 50 tests

For the amplification of HPV specific sequences

CE IVD

In vitro diagnostic medical device
according to EU directive 98/79/EC

1. Intended use

The VisionArray HPV Primer Kit 2.0 is intended to be used to amplify and biotinylate specific sections of the L1 region of the human papillomavirus (HPV) genomes by polymerase chain reaction (PCR).

The VisionArray HPV Primer Kit 2.0 is designed to amplify HPV types including but not limited to those detected by the corresponding VisionArray HPV Chips and genomic sequences of the human HLA-DQA1 gene as a PCR positive control.

The VisionArray HPV Primer Kit 2.0 has to be used with the VisionArray Detection Kit, the VisionArray PreCise Taq DNA Polymerase and the corresponding VisionArray HPV Chips. The automated analysis has to be performed with the VisionArray Analysis Package.

This product is designed for *in vitro* diagnostic use (according to EU directive 98/79/EC). Interpretation of results must be made within the context of the patient's clinical history with respect to further clinical and pathologic data of the patient by a qualified pathologist.

2. Clinical relevance

Refer to the instruction for use of the respective chip.

3. Test principle

By polymerase chain reaction (PCR), DNA sequences can be amplified selectively. The basic principle of the PCR is based on a recurring circle of 3 steps: denaturation, annealing and elongation. Repetition of these steps leads to an exponential amplification of the target sequences.

The first step of each cycle is the denaturation, where heating of the reaction mix leads to DNA single strands. During the annealing, complementary primers bind to the single stranded DNA. The primers flank the target sequence and serve as starting point for the integration of nucleotides during the phase of elongation, creating identical copies of the template DNA. The primers used in this kit are labelled with a biotin molecule. Hence, each new PCR product is automatically biotinylated, which later enables antibody detection.

The HPV Primer Mix 2.0 is a refinement of the GP5/GP6 system (Snijders et al., 1990) and is directed against the L1 gene, a highly conserved region of the HPV genome. Depending on the HPV genotype, the amplification results in PCR products of 139-148 bp fragment length.

Primers against the human HLA-DQA1 gene are recommended as a positive control by the WHO in their "Human Papillomavirus Laboratory Manual" and are therefore also included in the HPV Primer Mix 2.0.

In order to avoid contamination with PCR amplification products, uracil nucleotides are included into the HPV Primer Kit 2.0. By performing an Uracil-DNA-Glycosylase step prior to the PCR (recommended by the manufacturer) all sequences that contain uracil bases and therefore possible contaminations with PCR products from previous VisionArray PCRs can be removed. The Uracil-DNA-Glycosylase is inactivated by temperatures above 95°C so that the PCR reaction can be performed as usual.

4. Reagents provided

The following components are included:

Code	Components	Tests	Container
PR-0001-50	HPV Primer Mix 2.0	Σ 50	Screw-cap bottle (skirted)
NU-0001-50	dNTP/dUTP Solution	Σ 50	Screw-cap bottle (skirted)
	Instruction for use	1	

5. Materials required but not provided

Reagents:

- PCR chemicals (*Taq* Polymerase including reaction buffer, $MgCl_2$)

Note: The performance of the VisionArray system was validated using the VisionArray PreCise Taq DNA Polymerase (VE-0001). *The usage of other Taq polymerases need to be validated by the user.*

- Uracil-DNA-Glycosylase (recommended: VisionArray Uracil-DNA Glycosylase (VE-0002))
- H_2O (PCR-grade)
- VisionArray Detection Kit (VK-0003)

Equipment:

- PCR vessels
- Thermal cycler
- Pipettes
- VisionArray HPV Chips (VA-0001; VA-0002)
- VisionArray Analysis Package (E-4060)

6. Storage and handling

The components of the kit must be stored at -16...-22°C in an upright position. If these storage conditions are followed, the kit will function, without loss of performance, at least until the expiry date printed on the label.

The VisionArray HPV Primer Kit 2.0 is shipped at 2...8°C but should be returned to storage conditions as soon as possible.

The time period of the PCR product at room temperature should be as short as possible.

Repeated thawing/freezing of the HPV Primer Mix 2.0 and dNTP/dUTP Solution can lead to an impaired PCR efficiency and must therefore be avoided.

7. Warnings and precautions

- Read the instruction for use prior to use!
- Do not use the reagents after the expiry date has been reached!
- Avoid any cross-contamination and micro bacterial contamination of the reagents.
- Never pipet solutions with your mouth!
- A material safety data sheet is available on request for the professional user.
- A room separation of working steps with and without DNA as well as using clean benches for preparation of the PCR master mix is necessary to avoid contaminations.

8. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- Interpretation of results must be made within the context of the patient's clinical history with respect to further clinical and pathologic data by a qualified pathologist.
- The kit components are thoroughly adjusted to each other and the substitution of one or more components can lead to performance errors.
- It is important to use the indicated amounts of the components in order to avoid impairments of the reaction process.
- Repeated thawing and freezing of the DNA samples can lead to an impairment of the detection reaction.

9. Interfering substances

- Low PCR efficiency due to PCR inhibition in DNA raw material (e.g. blood).
- High concentrations of EDTA in Elution buffers may lead to an inhibition of the PCR.

10. Preparatory treatment of specimens

Formalin-fixed, paraffin-embedded (FFPE) tissue samples, cervical swabs/brush specimens, as well as ThinPrep specimens can be used as starting material for HPV genotyping.

Recommended DNA extraction kits:

- FFPE samples: Vision*Array* FFPE DNA Extraction Kit (VI-0001).
- ThinPrep samples: Vision*Array* Cytology DNA Extraction Kit (VI-0002)

After extraction, a measurement of the DNA concentration is necessary in order to check the quality and quantity of the DNA. Each sample should have a DNA concentration of at least 15 ng/µl with a high degree of purity (260/280: ~1.8).

Avoid DNA contaminations during the extraction procedure. When using a microtome, the tissue sections should be placed immediately in a reaction tube after cutting. The microtome blade should be changed between different tissue samples. The same applies for already fixated tissue samples mounted on glass slides. The scraper should be changed between different samples.

11. Preparatory treatment of the device

As a first step, determine the amount of required PCRs (n), which arises from the amount of DNA samples plus a negative control (reaction mixture without DNA template).

Pipetting scheme:

No.	Reagents	1x (final conc.)	nx
1	10x PCR Buffer*	2.5 µl	
2	$MgCl_2$ (25 mM)*	8.0 µl	
3	dNTP/dUTP Solution	1.0 µl	
4	HPV Primer Mix 2.0	1.0 µl	
5	Uracil-DNA Glycosylase (10 U/µl)**	0.05 µl	
6	*Taq* Polymerase (5 U/µl)*	0.3 µl	
7	Sample DNA	2.5-5.0 µl	
8	H_2O	ad 25 µl	
	Total Volume	**25 µl**	

*Mandatory: Vision*Array* PreCise Taq DNA Polymerase (VE-0001) incl. 10x PCR Buffer and $MgCl_2$

**Recommended: Vision*Array* Uracil-DNA Glycosylase (VE-0002)

- Handle all components according to the guidelines of the manufacturer. Thaw the components **3** and **4** on ice.
- Prepare a master mix (for the total volume) of the reagents No. **1-6** in the order of numeration of the pipetting scheme, mix gently and centrifuge briefly.
- Aliquot the master mix into DNA/DNase free PCR vials.
- Pipette the sample DNA into the master mix (No. **7** in the pipetting scheme). For the negative control add 5 µl DNA/DNase free water.
- If necessary, add water to reach the final reaction volume of 25 µl (No. **8** in the pipetting scheme).
- Transfer the samples into a prewarmed and calibrated thermal cycler.

12. Assay procedure

The amplification protocol described in this manual has been established in 0.2 ml PCR vials using the recommended enzymes on a Biometra TProfessional Thermocycler System. If necessary, modifications according to the manufacturer may be carried out when other thermal cyclers are used. This protocol has therefore to be tested for compatibility prior to use. The used thermal cycler has to be calibrated in accordance with the manufacturer´s guidelines.

Thermal profile:

Time	Temperature	Repeats	Step
10 min	25°C	x1	Uracil-DNA Glycosylase Incubation
10 min	95°C	x1	Activation of the HotStart *Taq* Polymerase, Deactivation of the Uracil-DNA Glycosylase
20 s	95°C	x10	Denaturation
30 s	55°C		Annealing
80 s	60°C		Elongation
20 s	95°C	x35	Denaturation
30 s	38°C		Annealing
80 s	60°C		Elongation
1 min	95°C	x1	Denaturation
∞	8°C	x1	

Ramping time: Δ 5°C/s

The thermal profile is optimised for the reagents recommended in this manual. Changes in the chemical composition or set up have to be validated by the user prior to use.
Once the PCR has finished, the reaction vial should be stored at -16°C...-22°C.

13. Interpretation of results

The Vision*Array* HPV Primer Kit 2.0 is intended to be used with the Vision*Array* HPV Chips and Vision*Array* Detection Kit. The interpretation of the results has to be made with the help of the Vision*Array* Analysis Package.

14. Recommended quality control procedures

In order to monitor correct performance of processed specimens and test reagents, each assay should be accompanied by external validated positive and negative control specimens. If internal and/or external controls fail to demonstrate appropriate staining, results with patient specimens must be considered invalid.

The control of the PCR and amplificates can be performed afterwards by separation in an agarose gel electrophoresis. The fragment length of the HPV types is around 140 bp and is only present in an HPV positive sample. The positive control shows a band at 227 bp.

Due to the low annealing temperature and PCR conditions that favour single stranded products, clearly delimited bands are not present in every test. However, a successful chip hybridization is still possible. Only the complete absence of a band in the gel indicates a failed PCR. See the troubleshooting section for further details.

15. Performance characteristics

Refer to the performance characteristics of the respective Vision*Array* HPV Chip.

16. Disposal

The disposal of reagents must be carried out in accordance with local regulations.

17. Troubleshooting

Any deviation from the operating instructions can lead to impairment of the detection reaction of the target sequence.

Problem	Possible cause	Action
Missing or little amplification product	Expired or degenerated PCR reagents; wrong thermal cycler program.	Check PCR reagents and thermal cycler program.
	Degraded template DNA; low DNA yield.	Store the DNA at -16…-22°C; avoid repeated thawing and freezing; use alternative extraction protocol.
	PCR inhibitors in the reaction mix.	Use alternative extraction protocol; dilute the sample with H_2O (PCR-grade) to the recommended DNA concentration before performing a PCR.
PCR amplificates in the negative control	Contamination of the reagents during sample preparation or in the PCR setup.	Use fresh reagents; avoid sample contamination; perform an Uracil-DNA-Glycosylase step in ahead of the PCR amplification.

18. Literature

- IARC Monographs on the evaluation of carcinogenic risks to humans, Vol. 100, 2012; ISBN 978 92 832 1319 2
- WHO Human Papillomavirus Laboratory Manual, First edition, 2009.
- Snijders P. J. F., et al. (1990) *Journal of General Virology* **71**:173-181.

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