ZYTOVISION

VisionArray®

VisionArray

HPV Primer Kit

REF VP-0001-50 Σ 50 tests

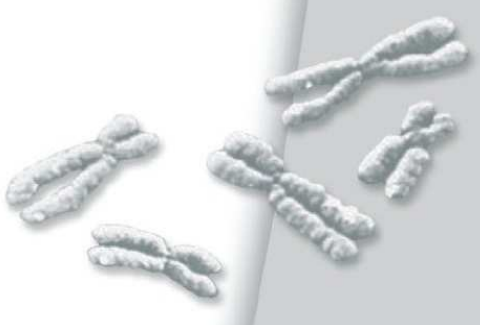
For the amplification of HPV specific sequences

CE

IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC



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Manufactured by:

42 life sciences GmbH & Co. KG
Fischkai 1
27572 Bremerhaven/ Germany
Phone: +49 471 4832-500
Fax: +49 471 4832-308
www.42ls.com
Email: info@42ls.com

Distributed by:

ZytoVision GmbH
Fischkai 1
27572 Bremerhaven/ Germany
Phone: +49 471 4832-300
Fax: +49 471 4832-509
www.zytovision.com
Email: info@zytovision.com

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1 Scope of Application

The Vision*Array* HPV Primer Kit is intended to be used to amplify and biotinylate specific sections of the L1 region of the Human Papilloma Virus (HPV) genomes by polymerase chain reaction (PCR).

The Vision*Array* HPV Primer Kit is designed to amplify HPV types including but not limited to those detected by the corresponding Vision*Array* HPV Chip and genomic sequences of the human HLA-DQA1 gene as a PCR positive control.
The Vision*Array* HPV Primer Kit has been developed to be used with the Vision*Array* Detection Kit and the corresponding Vision*Array* HPV Chip. The automated analysis has to be performed with the Vision*Array* 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 Basic Principles

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 Vision*Array* HPV Primer Mix 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 Papilloma Virus Laboratory Manuals* and are therefore also included in the Vision*Array* HPV Primer Mix.

In order to avoid contamination with PCR amplification products, uracil nucleotides are included into the Vision*Array* HPV Primer Kit. By performing a 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 Vision*Array* 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.

3 Safety Precautions and Disposal

- ✓ Read the operating instructions 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 pipette solutions with your mouth!
- ✓ The disposal of reagents must be carried out in accordance with local regulations!
- ✓ A material safety data sheet is available on request for the professional user!

4 The Vision*Array* HPV Primer Kit

4.1 Components

The following components are included:

Code	Components	Tests	Container
PR-0001-50	HPV Primer Mix	Σ 50	Screw-cap bottle (skirted)
NU-0001-0.05	dNTP/dUTP Solution	Σ 50	Screw-cap bottle (skirted)
	Instruction manual	1	

The components are sufficient for 50 tests.

4.2 Storage and Shelf Life

The components of the kit must be stored at -18...-22°C. If these storage conditions are followed, the kit will function, without loss of performance, at least until the expiry date printed on the label.

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

4.3 Test Material

The Vision*Array* HPV Primer Mix has been developed for the amplification of HPV types in DNA samples from formalin-fixed, paraffin-embedded tissues and cytologic specimens.

In order to perform a successful PCR amplification, the starting material (the extracted DNA) must be free from PCR inhibitors. Furthermore, we suggest that the samples should contain at least 15 ng/µl DNA with a high degree of purity (260/280: ~1.8).

4.4 Additional Material

- Chemicals:
    - PCR chemicals (*Taq* Polymerase including reaction buffer, $MgCl_2$)
    - Uracil-DNA-Glycosylase
    - H_2O (PCR-grade)
- Equipment:
    - PCR vessels
    - Thermal cycler
    - Pipettes
- The Vision*Array* HPV Primer Kit has to be used together with the Vision*Array* Detection Kit (Article-No. VK-0003) for the Vision*Array* HPV Chip (Article-No. VA-0001) and must be analyzed with the Vision*Array* Analysis Package (Article-No. E-4060).

The Vision*Array* Analysis Package must contain a Vision*Array* HPV Chip File for a successful scan.

4.5 Important Information

The following should be kept in mind:

- ✓ The amplificates that have been produced by the PCR are a contamination risk for subsequent tests. The subsequent steps should be performed spatially separated from the sample preparation and the PCR setup.
- ✓ We recommend using Uracil-DNA-Glycosylase in order to avoid contamination from previous studies.
- ✓ Deviations from the recommended amount of each component used can lead to unspecific amplification or impairment of the PCR.
- ✓ Repeated thawing/freezing of the Primer Mix and the dNTP/dUTP Solution can lead to an impaired PCR efficiency and must therefore be avoided.
- ✓ In each PCR amplification at least one negative control should be carried along in order to ensure the reliability of the test.

✓ The Vision*Array* HPV Primer Mix includes a PCR positive control (HLA-DQA1 control) that gives insights about the quality of the DNA and PCR. The positive control leads to two signals on the corresponding Vision*Array* HPV Chip when scanned with the Vision*Array* Analysis Package.

5 The Vision*Array* HPV Primer Protocol

5.1 Preparatory Steps

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 (1x)	
2	$MgCl_2$ (25 mM)*	8.0 µl (8 mM)	
3	dNTP/dUTP Solution	1.0 µl	
4	Primer Mix	1.0 µl (n/a)	
5	Uracil-DNA Glycosylase (10 U/µl)**	0.05 µl (0,5 U)	
6	*Taq* Polymerase (5 U/µl)*	0.3 µl (1,5 U)	
7	Sample DNA	up to 10 µl	
8	H_2O	ad 25 µl	
	Total Volume	**25 µl**	

*Recommended: Applied Biosystems AmpliTaq Gold 360 DNA Polymerase (# 4398833) incl. 10x PCR Buffer and $MgCl_2$
**Recommended: Bioron Uracil-DNA Glycosylase (#111025)

1. Handle all components according to the guidelines of the manufacturer. Thaw the components **3** and **4** on ice.
2. 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.
3. Aliquot the master mix into DNA/DNase free PCR vials.
4. Pipette the sample DNA into the master mix (No. **7** in the pipetting scheme). For the negative control add 10 µl DNA/DNase free water.
5. If necessary, add water to reach the final reaction volume of 25 µl (No. **8** in the pipetting scheme).
6. Transfer the samples into a prewarmed and calibrated thermal cycler.

5.2 PCR

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 -18°C…-22°C.

5.3 Agarose Gel Electrophoresis

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.

6 Interpretation of the results

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

7 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.

8 Problems and Possible Causes

Every deviation from the operating instructions can lead to impairment of the PCR and therefore missing or weak amplification 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 -20°C; Avoid repeated thawing and freezing; Use alternative extraction protocol.
	PCR inhibitors in the reaction mix.	Use alternative extraction protocol.
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.

9 Explanation of the Symbols

	Check information material
	Check instruction for use
	Manufacturer
	Content sufficient for n tests
	LOT number
	Product code
	Use until
	Allowed storing temperature
	Store in the dark