

## VisionArray® Arrays for DNA analysis

The VisionArray HPV Primer Kit is intended to be used to amplify and biotinylate specific sections of the L1 region of the HPV genomes by PCR. The VisionArray HPV Primer Kit should be used in combination with the VisionArray Detection Kit and the corresponding VisionArray HPV Chip. The automated analysis has to be performed with the VisionArray Analysis Package.

### 1) Preparatory Steps

- Determine the amount of required PCRs (n)

No.	Reagents	Final conc. (1x)
1	10x PCR Buffer*	2.5 µl (1x)
2	MgCl <sub>2</sub> (25 mM)*	8 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 <sub>2</sub> O	Ad 25 µl
<b>Total Volume</b>		<b>25 µl</b>

\* Recommended: Applied Biosystems AmpliTaq Gold 360 DNA Polymerase incl. 10x PCR Buffer and MgCl<sub>2</sub>  
 \*\* Recommended: Bioron Uracil-DNA Glycosylase

- Thaw components 3 & 4 on ice
- Prepare a master mix of reagents 1-6
- Mix gently and centrifuge briefly
- Aliquot the master mix into DNA/DNase free PCR vials
- Pipette the sample DNA (7) into the master mix
- For the negative control add 10 µl DNA/DNase free water
- Transfer the samples into a prewarmed and calibrated thermal cycler

This is a condensed protocol for the VisionArray® HPV Primer Kit and should not replace the instruction for use!

VisionArray HPV Primer Kit

## VisionArray® Arrays for DNA analysis

### 2) PCR

- The amplification protocol has been established for the Biometra TProfessional Thermocycler System

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

Ramping time: Δ 5 °C/s

- Once PCR has finished, the product should be stored at -18...-22 °C

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