

VisionArray® Arrays for DNA analysis

The VisionArray HPV PreCise Master Mix, the VisionArray HPV Primer Kit 2.0, the VisionArray PreCise Taq DNA Polymerase and the VisionArray Uracil-DNA Glycosylase are intended to be used to amplify and biotinylate specific sections of the L1 region of the HPV genomes by PCR. For the detection procedure the VisionArray Detection Kit should be used in combination with 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 PCR reactions (n)

Reagents (final conc. n=1)		HPV PreCise Master Mix (1.5 µl)
(1) 10x PCR Buffer	2.5 µl (1x)	Included
(2) MgCl ₂ (25 mM)	8 µl (8 mM)	Included
(3) dNTP/dUTP Solution	1.0 µl	Included
(4) Primer Mix 2.0	1.0 µl (n/a)	Included
(5) VisionArray Uracil-DNA Glycosylase	0.05 µl (0.5 U)	Included
(6) VisionArray PreCise Taq DNA Polymerase	0.3 µl (1.5 U)	Included
(7) Sample DNA	2.5-5 µl	2.5-5 µl
(8) H ₂ O	Ad 25 µl	Ad 25 µl
Total Volume	25 µl	25 µl

- 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

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2) PCR

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

Time	Temperature	Repeats	Step
10 min	25 °C	1x	Uracil-DNA Glycosylase Incubation
10 min	95 °C	1x	Activation: HotStart Taq Polymerase Deactivation: Uracil-DNA Glycosylase
20 s	95 °C		Denaturation
30 s	55 °C	10x	Annealing
80 s	60 °C		Elongation
20 s	95 °C		Denaturation
30 s	38 °C	35x	Annealing
80 s	60 °C		Elongation
1 min	95 °C	1x	Denaturation
∞	8 °C	1x	
Ramping time: Δ 5 °C/s			

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

Alternatively, the VisionArray HPV PreCise Master Mix can be used

This is a condensed protocol for the VisionArray HPV PreCise Master Mix and VisionArray® HPV Primer Kit 2.0 by using the VisionArray® PreCise Taq DNA Polymerase and the VisionArray® Uracil-DNA Glycosylase and should not replace the instruction for use!

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