VisionArray
FUNGI PreCise Master Mix 1.0

REF ES-0009-50 Σ 50 tests

For the amplification of fungi specific sequences

For research use only.
Not for use in diagnostic procedures.

1. Intended purpose

The VisionArray FUNGI PreCise Master Mix 1.0 is intended to be used to amplify and biotinylate specific sections of the 16S-23S ITS region of fungi genomes by polymerase chain reaction (PCR).

The VisionArray FUNGI PreCise Master Mix 1.0 is designed to amplify fungi types including but not limited to those detected by the corresponding VisionArray FUNGI Chips and genomic sequences of the human HLA-DQA1 gene as a PCR positive control.

The VisionArray FUNGI PreCise Master Mix 1.0 has to be used with the VisionArray Detection Kit and the corresponding VisionArray FUNGI Chips. The automated analysis has to be performed with a VisionArray Software.

2. Test principle

DNA-fragments with a specific sequence are detected from a pool of DNA-fragments on a glass chip with the help of immobilized DNA capture sequences by DNA/DNA-hybridization. For this detection system, DNA-samples from formalin-fixed, paraffin-embedded tissue or cell samples can be used as raw material. As a first step, the target sequences in these samples have to be amplified and biotinylated by PCR. The hybridization between the amplified sequences and the complementary DNA capture sequences is performed subsequently. After the hybridization, the unspecifically bound DNA is washed away by short stringent wash steps. The specifically bound biotinylated sequences are secondary labelled with a streptavidin-peroxidase-conjugate afterwards and visualized by tetramethylbenzidine (TMB) staining.

3. Reagents provided

The VisionArray FUNGI PreCise Master Mix 1.0 is composed of:

- VisionArray FUNGI Primer
- PreCise Taq DNA Polymerase
- Uracil-DNA Glycosylase
- H_2O
- $MgCl_2$
- PCR-Buffer
- dNTP/dUTP Solution

The VisionArray FUNGI PreCise Master Mix 1.0 is available in one size:

- ES-0009-50: 0.75 ml (50 reactions of 15 µl each)

4. Materials required but not provided

Reagents:
- H_2O (PCR-grade)
- VisionArray Detection Kit (VK-0003)

Equipment:
- PCR vessels
- Thermal cycler
- Pipettes
- VisionArray FUNGI Chips (VA-0006)
- VisionArray SingleScan Software (E-4301) or VisionArray MultiScan Software (E-4302)

5. Storage and handling

The VisionArray FUNGI PreCise Master Mix 1.0 must be stored at -16...-22°C in an upright position. If these storage conditions are followed, the product will function, without loss of performance, at least until the expiry date printed on the label.
Minimize the number of freeze-thaw cycles to a maximum of 10 cycles by storing in working aliquots. After opening the vial, use the device within 6 months.
The time period of the PCR product at room temperature should be as short as possible. Return to storage conditions immediately after use. Do not use reagents beyond expiry date indicated on the label. The product is stable until expiry date indicated on the label when handled accordingly.

6. Warnings and precautions

- Read the instructions for use prior to use!
- Do not use the products after the expiry date has been reached!
- Please check if packaging is intact before use, do not use product if packaging is damaged.
- Report any serious incident that has occurred in relation to the product to the manufacturer and the competent authority according to local regulations!
- If reagents come into contact with skin, rinse skin immediately with copious amounts of water!
- A material safety data sheet is available on request for the professional user.
- Do not reuse products, unless reuse is explicitly permitted!
- Avoid cross-contamination of samples as this may lead to erroneous results.
- 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.
- Chips should be used in a dust-free setting. Avoid the contamination of the chip surface with dust or other particles!
- Avoid direct contact with the array field on the chip-surface!
- Only the labelled side of the slide can be used for hybridization.

Hazard and precautionary statements:

This product is not classified as hazardous according to Regulation (EC) No. 1272/2008.

7. Limitations

- For research use only.
- For professional use only.
- For non-automated use only.
- 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.

8. Interfering substances

- Low PCR efficiency due to PCR inhibitors in DNA raw material (e.g. blood).
- High concentrations of EDTA in DNA elution buffers may lead to an inhibition of the PCR. Use only the recommended amounts of DNA.
- Use of PCR additives that could influence the hybridization (e.g. DMSO, betaine, urea).

9. Preparation of specimens

Formalin-fixed, paraffin-embedded (FFPE) tissue samples can be used as starting material for fungi genotyping.

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.

10. 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	Vision*Array* FUNGI PreCise Master Mix	15 µl (1x)	
2	Sample DNA	2.5-5 µl	
3	H_2O	to 25 µl	
	Total Volume	**25 µl**	

- Aliquot the Vision*Array* FUNGI PreCise Master Mix 1.0 into DNA/DNase free PCR vials.
- Pipette the sample DNA into the Master Mix (No. **2** in the pipetting scheme). For the negative control add 10 µl DNA/DNase free water.
- If necessary, add water to reach the final reaction volume of 25 µl (No. **3** in the pipetting scheme).
- Transfer the samples into a prewarmed and calibrated thermal cycler.

11. 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.

12. Interpretation of results

The Vision*Array* FUNGI PreCise Master Mix 1.0 is intended to be used with a Vision*Array* FUNGI Chip and Vision*Array* Detection Kit. The interpretation of the results has to be made with the help of the respective Vision*Array* Software.

13. 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 fungi types is around 141-478 bp and is only present in a fungi positive sample. The positive control shows a band at 227 bp.

Due to the 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.

14. Performance characteristics

Please refer to the performance characteristics of the respective Vision*Array* FUNGI Chip.

15. Disposal

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

16. 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…-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.

17. Literature

- Kidd S, et al. (2023) *Open Forum Infect Dis*;10(1):ofac559. doi: 10.1093/ofid/ofac559. PMID: 36632423; PMCID: PMC9825814.
- Schoch CL, et al. (2012) *Proc Natl Acad Sci U S A.*;109(16):6241-6. doi: 10.1073/pnas.1117018109. PMID: 22454494; PMCID: PMC3341068.

18. Revision

www.zytovision.com

Please refer to www.zytovision.com for the most recent instructions for use as well as for instructions for use in different languages.

Our experts are available to answer your questions.
Please contact helptech@zytovision.com

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