Vision*Array*
MYCO PreCise Master Mix 2.0

REF ES-0008-50 Σ 50 tests

For the amplification of mycobacterial specific sequences

CE IVD

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

1. Intended use

The Vision*Array* MYCO PreCise Master Mix 2.0 is intended to be used to amplify and biotinylate specific sections of the ITS and, in case of the M. tuberculosis complex, the IS6110 region and, in case of M. chimaera, the SR4 region (Zozaya-Valdés et al 2017) of mycobacterial genomes by polymerase chain reaction (PCR) using DNA samples extracted from e.g. clinical specimens, pulmonary smears or cultivated samples.

The Vision*Array* MYCO PreCise Master Mix 2.0 is designed to amplify mycobacteria including but not limited to those detected by the corresponding Vision*Array* MYCO Chips and, if present in the DNA sample, genomic sequences of the human HLA-DQA1 gene as a PCR positive control.

The Vision*Array* MYCO PreCise Master Mix 2.0 has to be used with the Vision*Array* Detection Kit and the corresponding Vision*Array* MYCO Chips. The automated analysis has to be performed with a Vision*Array* Software.

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 instructions 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.

In order to avoid contamination with PCR amplification products, uracil nucleotides are included into the Vision*Array* MYCO PreCise Master Mix 2.0. By performing a Uracil-DNA-Glycosylase step prior to the PCR 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.

4. Reagents provided

The Vision*Array* MYCO PreCise Master Mix 2.0 is composed of:

- Vision*Array* MYCO Primer
- Taq DNA Polymerase
- Uracil-DNA Glycosylase
- H_2O
- $MgCl_2$
- PCR-Buffer
- dNTP/dUTP Solution

The Vision*Array* MYCO PreCise Master Mix 2.0 is available in one size:

- ES-0008-50: 0.75 ml (50 reactions of 15 µl each)

5. Materials required but not provided

Reagents:

- H_2O (PCR-grade)
- Vision*Array* Detection Kit (VK-0003)

Equipment:

- PCR vessels
- Thermal cycler
- Pipettes
- Vision*Array* MYCO Chip 2.0 (VA-0005)
- Vision*Array* SingleScan Software (E-4301) or Vision*Array* MultiScan Software (E-4302)

6. Storage and handling

The Vision*Array* MYCO PreCise Master Mix 2.0 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.

7. Warnings and precautions

- Read the instructions for use prior to use!
- Do not use the reagents after the expiry date has been reached!
- Please check if packaging is intact before use, do not use product if packaging is damaged.
- 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.
- 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 DNA elution buffers may lead to an inhibition of the PCR. Use only the recommended amounts of DNA.

10. Preparatory treatment of specimens

DNA samples extracted from e.g. clinical specimens, pulmonary smears or cultivated samples can be used as starting material for mycobacterial 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.

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	Vision*Array* MYCO PreCise Master Mix 2.0	15 µl	
2	Sample DNA	2.5-5 µl	
3	H_2O	ad 25 µl	
	Total Volume	**25 µl**	

- Aliquot the Vision*Array* MYCO PreCise Master Mix 2.0 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.

12. Assay procedure

The amplification protocol described in this manual has been established in 0.2 ml PCR vials using the Master Mix 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	x35	Denaturation
90 s	60°C		Annealing and Elongation
60 s	95°C	x1	Denaturation
∞	10°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* MYCO PreCise Master Mix 2.0 is intended to be used with a Vision*Array* MYCO Chip and Vision*Array* Detection Kit. The interpretation of the results has to be made with the help of the respective Vision*Array* Software.

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 mycobacterial species is 212 – 314 bp for the sections of the ITS region, 122 bp for the IS6110 region of M. tuberculosis complex and 139 bp for the SR4 region of M. chimaera. The positive control shows a band at 227 bp.

Due to PCR conditions that favour single stranded products, clearly delimited bands are not present in every test. However, a successful chip hybridization is still possible. See the troubleshooting section for further details.

15. Performance characteristics

Refer to the performance characteristics of the respective Vision*Array* MYCO 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…-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.

18. Literature

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- Oren A. and Garrity G. (2018) Int J Syst Evol Microbiol 68:1411–1417.
- Zozaya-Valdés E., et al (2017) J Clin Microbiol 55:1847–1856.

Our experts are available to answer your questions.
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