




VisionArray MYCO Sequencing Primers

REF PR-0003-50  50

For further characterization of Mycobacteria specific sequences from VisionArray PCR products

For Research Use Only.
Not for use in diagnostic procedures

1. Intended use

The VisionArray MYCO Sequencing Primers (PR-0003-50) are intended to be used for further characterization of Mycobacteria specific sequences from VisionArray PCR products.

2. Clinical relevance


This product is for research use only and not intended for diagnostic procedures.

3. Test principle

The VisionArray MYCO Sequencing Primers (PR-0003-50) add sequencing adaptors to VisionArray MYCO PCR products, allowing further characterization thereof by Sanger sequencing. The PCR product obtained from the VisionArray MYCO PreCise Master Mix 2.0 serves as template in a nested re-amplification of the mycobacterial ITS region, adding adaptor sequences for the commonly used sequencing primers SP6 and T7. The PCR product obtained can then be analyzed in a subsequent sequencing reaction by appropriate providers and the received sequences further characterized by the customer using BLAST or other sequence analysis software.

4. Reagents provided

The following components are included:

Code	Component	Tests	Container
PR-0003-50	<u>VisionArray MYCO Sequencing Primers</u>	 50	Screw-cap bottle
	Instructions for use	1	

The VisionArray MYCO Sequencing Primers are available in one size:

- PR-0003-50: 50 µl (50 reactions of 1 µl each)

5. Materials required but not provided

Reagents:

- PCR chemicals (*Taq* Polymerase including reaction buffer, MgCl₂)
- dNTP/dUTP-Solution
- H₂O (PCR-grade)
- PCR product obtained from the VisionArray MYCO PreCise Master Mix 2.0 (ES-0008)

Equipment:

- PCR vessels
- Thermal cycler
- Pipettes

6. Storage and handling

The VisionArray MYCO Sequencing Primers must be stored at -16°C...-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 product 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!
- Avoid any cross-contamination and micro-bacterial contamination of the reagents!
- 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! Keep in mind that amplified PCR product is used as template in this reaction, requiring extra precaution!
- Do not use Uracil-DNA Glycosylase in order to avoid degradation of the Uracil-containing template!
- Never pipet solutions with your mouth!

Hazard and precautionary statements for PR-0003

This mixture is not classified as hazardous in accordance with Regulation (EC) No. 1272/2008.

8. Limitations

- For research use only.
- For professional use only.
- Repeated thawing and freezing of the DNA samples can lead to an impairment of the detection reaction.

9. Interfering substances

Not applicable.

10. Preparation of specimens

Use only the PCR product obtained from VisionArray MYCO PreCise Master Mix 2.0 (ES-0008) as template.

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	H ₂ O (PCR-grade)	15,2 µl	
2	10x PCR buffer	2,5 µl	
3	25 mM MgCl ₂	4 µl	
4	dNTP/dUTP Solution (10 mM each ATCG, 20 mM U)	1 µl	
5	VisionArray MYCO Sequencing Primers	1 µl	
6	TAQ 5U/µl	0,3 µl	
7	Nested template (VisionArray MYCO PCR product)	1 µl	
	Total volume	25 µl	

- Handle all components according to the guidelines of the manufacturer. Thaw the components **4** and **5** on ice.
- 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 a Biometra TProfessional Thermocycler System. If necessary, modifications according to the manufacturer may be carried out when other thermal cyclers are used. This protocol therefore has 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
5 min	95°C	x1	Activation of the <i>Taq</i> Polymerase,
30 s	95°C	x35	Denaturation
30 s	62°C		Annealing
30 s	60°C		Elongation
5 min	60°C	x1	Final elongation
∞	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 until further processing.

13. Interpretation of results

The PCR product obtained can be sequenced with help of the widely used sequencing primers T7 and SP6. For enhanced precision, we recommend sequencing both strands in two separate reactions using T7 and SP6, respectively. While the T7 anchor sequence is attached to the forward VisionArray MYCO Sequencing Primer, the SP6 anchor is attached to the reverse primer. For shipping conditions and further details/requirements of the sequencing process, e.g. PCR purification or sequencing template amount, please contact your sequencing service provider.

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14. Recommended quality control procedures

Verification of the PCR and amplicates obtained can be performed afterwards by separation of an aliquot by agarose gel electrophoresis. The expected fragment length is depending on the mycobacterial species present in the sample. Fragment lengths of mycobacteria genotyped by the VisionArray MYCO Chip are within range of ~ 220 to ~ 320 bp. Mycobacteria not covered by the chip may deviate, depending on their ITS region. If multiple species of different fragment lengths were present in the sample, several contiguous bands or a diffuse band/smear may appear at the expected range.

15. Performance characteristics

This product is for research use only and not intended for diagnostic procedures.

16. Disposal

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

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



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