VisionArray Detection Kit

REF VK-0003-50 Σ 50 tests

For qualitative detection of DNA sequences on Vision*Array* Chips.

CE IVD

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

1. Intended use

The Vision*Array* Detection Kit has been developed to be used with a Vision*Array* PCR Primer Kit or a Vision*Array* PreCise Master Mix and the corresponding Vision*Array* DNA Chip for the qualitative detection of specific DNA sequences. The automated analysis has to be performed with the Vision*Array* Analysis Package.

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 instruction for use of the respective chip.

3. Test principle

Sequence-specific DNA fragments in a pool of DNA fragments are detected by DNA/DNA hybridization with immobilized DNA catchers on a glass chip. First, the target sequences in this material have to be amplified via PCR and simultaneously marked with biotin molecules. Subsequently, the amplified sequences hybridize with the complementary DNA catchers on the glass chip. After the hybridization, unspecifically bound DNA fragments are removed by short and stringent washing steps. The specifically bound and biotinylated sequences are visualized by secondary marking with a streptavidin-peroxidase conjugate and a staining with tetramethylbenzidine (TMB).

4. Reagents provided

The following components are included:

Code	Components	Amount	Container
HY-0001-1	Hybridization Solution	1 ml	Reaction vessel, red lid
WB-0012-250	100x Wash Buffer	250 ml	Screw-cap bottle (large)
AB-0016-5	Detection Solution	5 ml	Screw-cap bottle (small)
SB-0009-5	Blue Spot Solution	5 ml	Screw-cap bottle (small), brown
	Instructions for use	1	

The Hybridization Solution, Detection Solution, and Blue Spot Solution are sufficient for 50 reactions. The 100x Wash Buffer is sufficient for 50 tests with 6 staining jars of 70 ml each.

5. Materials required but not provided

Reagents:

- PCR product created with a Vision*Array* Primer Kit or a Vision*Array* PreCise Master Mix
- Deionized or distilled water

Equipment:

- Vision*Array* Analysis Package (E-4060)
- Vision*Array* DNA Chips
- Hybridizer or hybridization oven with humidity chamber
- Slide centrifuge
- Staining jars, 50-80 ml
- Pipettes

Note: The VisionArray Analysis Package must contain the corresponding VisionArray Chip File for a successful scan.

6. Storage and handling

The components of the kit must be stored at 2...8°C in an upright position. Store the Blue Spot Solution protected from light. If these storage conditions are followed, the kit will function, without loss of performance, at least until the expiry date printed on the label.

7. Warnings and precautions

- Read the instruction 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.
- Some of the set components contain substances (in low concentrations and volumes) that are harmful to health. Avoid any direct contact with the reagents. Take appropriate protective measures (use disposable gloves, protective glasses, and lab garments)!
- If reagents come in contact with skin, rinse skin immediately with copious quantities of water!
- Never pipet solutions with your mouth!
- A material safety data sheet is available on request for the professional user.

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.
- The kit components are thoroughly adjusted to each other and the substitution of one or more components can lead to performance errors.
- 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.
- Do not work under laminar flow during the assay procedure since this might lead to an impairment of the results.

9. Interfering substances

- Low PCR efficiency due to PCR inhibition in DNA raw material (e.g. blood).
- Use of PCR additives that could influence the hybridization (e.g. DMSO, betaine, urea).

10. Preparatory treatment of specimens

Starting material for this detection system are DNA sequences that have been amplified and biotinylated with a Vision*Array* Primer Kit or a Vision*Array* PreCise Master Mix.

11. Preparatory treatment of the device

- Preparation of the 1x Wash Buffer: Dilute 1 part 100x Wash Buffer with 99 parts deionized or distilled water (in a closed container diluted 1x Wash Buffer is stable for one month at RT (18…22°C)).
- Bring Hybridization Solution, Detection Solution, Blue Spot Solution, and 1x Wash Buffer to RT (18…22°C). Possible precipitates in the Hybridization Solution must be solved by brief heating (max. 37°C).
- Heat the hybridizer or hybridization oven to 42°C prior to use.

12. Assay procedure

1 Remove the protective cover from the blue frames of the array field.

2 Preparation of the hybridization mix:

20 µl Hybridization Solution
\+ 10 µl PCR product
30 µl hybridization mix (enough for one chip)

Mix the hybridization mix thoroughly by pipetting up and down.

3 Pipette 30 µl of the hybridization mix carefully on the left side of the array field (with label on the right) avoiding trapped air bubbles. Coat the whole array field by carefully covering the array field from the left to the right side with the supplied plastic lid.

4 Transfer the chip quickly to the pre-heated hybridizer or hybridization oven with humidity chamber and incubate 30 min at 42°C (+/- 1°C).

Note: This step should be done for each array one after the other, never in parallel. Deviations of more than 1°C should be avoided. We advise to use a calibrated thermometer.

5 Prepare 3 staining jars with 1x Wash Buffer in the meantime.

6 Once the incubation time is over, take the chip out of the incubator and remove the lid carefully. Drain off the hybridization mix carefully on a paper tissue and wash the slide immediately in 1x Wash Buffer. Therefore, gently agitate the slide 3 times bidirectional in the first staining jar. Repeat this washing procedure in the 2nd staining jar. Afterwards, transfer the chip into the 3rd staining jar, agitate 3 times and incubate for 1 min.

Note: Do not use more than 6 slides per staining jar. *Not handled slides should remain at hybridization temperature. Exposure to room temperature should be as short as possible.*

7 Take the chip out of the staining jar, drain it shortly on a tissue and dry it by centrifugation in the slide centrifuge for 15-30 s.

Note: The usage of a slide centrifuge is absolutely mandatory in order to prevent droplets left on the array.

8 Pipette 100 µl Detection Solution carefully onto the dry array field without touching the surface. The array field has to be covered evenly and air bubbles have to be removed.

9 Incubate for 10 min on an even surface at RT (18…22°C).

10 In the meantime prepare 3 staining jars with 1x Wash Buffer.

11 After incubation, wash and dry as described in step 6 and 7. Keep the staining jar that was used last for step 13.

12 Apply 100 µl Blue Spot Solution carefully on the whole array field and incubate for 5 min at RT (18…22°C). The color development can be observed by visual inspection. In the case of a fast and heavy staining, the incubation can be stopped early.

Note: The Blue Spot Solution should be stored and incubated in the dark.

13 Wash off the Blue Spot Solution on the chip, in the 1x Wash Buffer staining jar from step 10, for approximately 15 sec.

14 Drain the chip shortly on a paper tissue and dry it by centrifugation in the slide centrifuge for 30 s.

The chips are now ready for analysis with the Vision*Array* Analysis Package.

13. Interpretation of results

13.1 General Note

With the help of the Vision*Array* DNA Chip it is possible to make a statement about the presence or absence of specific DNA sequences. The intensity of the signals is influenced by the frequency of the target sequences in the sample as well as by further factors of the detection system. It is not possible to use the absolute values of the signal intensity for the determination of the DNA concentration.

13.2 Evaluation

After following this protocol, the chip can be evaluated. Positive signals are visible on the slide as dark blue circular areas. The automated evaluation of the chip is performed with the Vision*Array* Analyzer Software.

13.3 Software-Based Evaluation

The automated evaluation of the results is performed by the Vision*Array* Analyzer Software. A comprehensive manual for a chip-analysis is enclosed to the 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.

15. Performance characteristics

Refer to the performance characteristics of the respective Vision*Array* DNA 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
No signal	Wrong temperature	Check the hybridization temperature
	Expired reagents	Check the reagents
Only guide dots and no other signals	Problems with the PCR product (PCR not efficient enough or DNA template degraded)	Check PCR efficiency with a positive control; Check PCR chemicals and thermal cycler program; Check PCR product in agarose gel
	Wrong raw material	Check the raw materials
	Wrong combination of chip and sample	Check the sample/chip combination
Only guide dots and PCR control, but no other signals	No target sequence present	Use positive control
Only guide dots and specific signals, but no positive control	Degraded sample	New DNA extraction; store at -16…-22°C
Too much background	Incubation time of Detection Solution or Blue Spot Solution too long; temperature during incubation too high	Check incubation time and temperature of Detection Solution and Blue Spot Solution
	Slides not properly dried	Check drying step
Strong, leaking signals	Incubation time of Detection Solution or Blue Spot Solution too long or temperature too high	Stepwise adjustment of the incubation time and temperature of Detection Solution and Blue Spot Solution
Weak signals	Hybridization temperature incorrect	Check temperature
	Hybridization time too short	Extend hybridization time to a maximum of 30 min
	Incubation time of Detection Solution or Blue Spot Solution too short	Extend incubation time of Detection Solution and Blue Spot Solution
	Weak PCR amplification/ bad quality of the DNA template	Check DNA template
Cross-hybridization signals, false positive signals	Contamination of the PCR chemicals or PCR product	Replace the PCR chemicals in use
	Contamination during the preparation of the PCR or of the hybridization mix	Avoid transfer of sample during the preparation of the mix
	Hybridization temperature too low	Check hybridization temperature
	Several chips incubated too long in the same wash buffer	Swift execution of the washing steps
Single signal instead of duplicates	Mechanical elimination of the second signal, e.g. due to contact with the pipette tip	Avoid direct contact with the array field
	Irregular covering of the array field due to air bubbles	Apply solutions without air bubbles
	Weak signals around the threshold (1 above and 1 below)	Repeat PCR and detection under consideration of the conditions required in the manual

Our experts are available to answer your questions.
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