ZYTOVISION

VisionArray®

VisionArray Detection Kit

REF VK-0003-50 Σ 50 tests

For qualitative detection of DNA sequences on VisionArray Chips

4250380M008PY

CE IVD

In vitro diagnostic medical device

according to IVDR (EU) 2017/746

1. Intended purpose

The VisionArray Detection Kit is intended to be used with a VisionArray PreCise Master Mix and the corresponding VisionArray DNA Chip for the qualitative detection of specific DNA sequences. The automated analysis has to be performed with a VisionArray Software.

The product is intended for professional use only. All tests using the product should be performed in a certified, licensed anatomic pathology laboratory under the supervision of a pathologist/human geneticist by qualified personnel.

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 labeled with a streptavidin-peroxidase-conjugate afterwards and visualized by tetramethylbenzidine (TMB) staining.

3. 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.

4. Materials required but not provided

Reagents:

- PCR product created with a VisionArray PreCise Master Mix
- Deionized or distilled water

Equipment:

- VisionArray SingleScan Software (E-4301) or VisionArray MultiScan Software (E-4302)
- VisionArray DNA Chips
- Hybridizer or hybridization oven with humidity chamber
- Slide centrifuge
- Staining jars, 50-80 ml
- Pipettes

5. 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 product will function, without loss of performance, at least until the expiry date printed on the label.
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!
- 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 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 labeled side of the slide can be used for hybridization.

Hazard and precautionary statements for HY-0001:

The hazard determining component is Formamide.

Danger

H351	Suspected of causing cancer.
H360FD	May damage fertility. May damage the unborn child.
H373	May cause damage to organs through prolonged or repeated exposure.
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P405	Store locked up.

Hazard and precautionary statements for AB-0016 and WB-0012:

The hazard-determining component is a reaction mass of: 5-chloro-2-methyl-2H-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	IF skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

7. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- For non-automated 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.
- The performance was validated using the procedures described in these instructions for use. Modifications to these procedures might alter the performance and have to be validated by the user. This IVD is only certified as CE when used as described in this instruction for use within the scope of the intended use.

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

Starting material for this detection system are DNA sequences that have been amplified and biotinylated with a VisionArray PreCise Master Mix.

10. 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.

11. 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* Software.

12. Interpretation of results

12.1 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.

12.2 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 respective Vision*Array* Software.

12.3 Software-Based Evaluation

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

14. Performance characteristics

Please refer to the performance characteristics of the respective Vision*Array* DNA 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
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

17. Revision

Revision	Description of the change
2.1.1	6. Warnings and precautions Added note for checking the integrity of the packaging

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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
For the summary of safety and performance, please refer to www.zytovision.com.

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