Vision*Array*

Detection Kit

REF VK-0003-50 Σ 50 tests

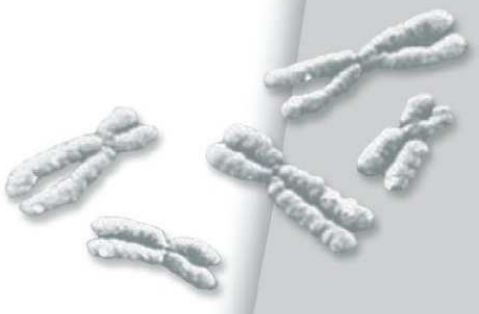
For qualitative detection of DNA-Sequences on Vision*Array* Chips.

CE

IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC



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Contents

1 Scope of Application

The Vision*Array* Detection Kit has been developed to be used with the Vision*Array* PCR Primer Kit and the corresponding Vision*Array* 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.

The Vision*Array* Detection Kit contains all reagents that are required for the simple and fast hybridization and detection of PCR products on Vision*Array* Chips.

The Vision*Array* Detection Kit contains a Hybridization Solution (HY-0001-1) that has to be mixed with the PCR products and subsequently applied on the Vision*Array* Chip. The Hybridization Solution contains a hybridization control that binds to the catchers in the guide dots of the array. Stained guide dots prove a successful hybridization and detection. Additionally, these dots provide orientation on the chip and are used for the calculation of the relative intensity of the signals. Furthermore, the Vision*Array* Detection Kit contains concentrated 100x Wash Buffer (WB-0012-250), a Detection Solution (AB-0016-5), and the Blue Spot Solution (SB-0009-5) for staining. All solutions with the exception of the wash buffer are ready for use.

2 Basic Principles

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).

3 Safety Precautions and Disposal

- ✓ Read the operating instructions 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 system 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 quantities of water!
- ✓ Never pipet solutions with your mouth!
- ✓ The disposal of reagents must be carried out in accordance with local regulations!
- ✓ A material safety data sheet is available on request for the professional user!

4 The Vision*Array* Detection Kit

4.1 Components

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.

4.2 Storage and Shelf Life

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.

4.3 Test Material

Only DNA sequences that have been amplified and biotinylated with the Vision*Array* Primer Kit are to be used as primary material for the Vision*Array* Detection Kit.

4.4 Additional Materials

The following reagents, materials and equipment are not included in the Vision*Array* Detection Kit:

Reagents and materials:

- PCR products created with the Vision*Array* Primer Kit
- Vision*Array* Chip
- Deionized or distilled water

Equipment:

- Hybridizer or hybridization oven with humidity chamber
- Slide-centrifuge
- Staining jars, 50-80 ml
- Pipettes
- Vision*Array* Analysis Package
- Vision*Array* Chip File

4.5 Important Information

The following should be kept in mind:

- ✓ The temperature for hybridization, described in the protocol, should be used in general. Deviations of more then 1°C should be avoided. We advise to use a calibrated thermometer.
- ✓ 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.

5 The Vision*Array* Detection Kit Protocol

5.1 Preparatory Steps

- 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).
- 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)).
- Heat the hybridizer or hybridization oven to 42°C prior to use.

5.2 Vision*Array* Hybridization and Detection

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 in the array field without touching the surface. The array field has to be coated completely with the solution and potential air bubbles have to be removed. Close the hybridization chamber afterwards carefully 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).

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.

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.

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

6 Interpretation of Results

6.1 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.

6.2 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.

7 Problems and Possible Causes

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all.

Problem	**Possible cause**	**Action**
No signal	Wrong raw material	Check the raw materials
	Wrong combination of chip and sample	Check the sample/chip combination
	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
Only guide dots and PCR control, but no other signals	No target sequence present	Use positive control
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
Strong, leaking signals	Incubation time of Detection Solution or Blue Spot Solution too long	Stepwise adjustment of the incubation time of Detection Solution and Blue Spot Solution
Weak signals	Hybridization temperature incorrect	Check temperature
	Hybridization time too short	Extend hybridization time
	Incubation time of Detection Solution or Blue Spot Solution too short	Extend incubation time of Detection Solution and Blue Spot Solution
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

8 Explanation of Symbols

	Check information material
	Check Instruction for use
	Manufacturer
	Content sufficient for n tests
	Batch number
	Product code
	Use until
	Allowed storing temperature
	Store in the dark