Vision*Array*
Cytology DNA Extraction Kit

REF VI-0002-50 Σ 50

For the DNA isolation from cell samples

For Research Use Only.
Not for use in diagnostic procedures

1 Intended use

The Vision*Array* Cytology DNA Extraction Kit (**VI-0002-50**) is intended to be used for the isolation of genomic DNA from liquid-based cytology samples.

2 Clinical relevance

This product is for research use only and should not be used for diagnostic procedures.

3 Test principle

The Vision*Array* Cytology DNA Extraction Kit is designed for the efficient isolation of genomic DNA from small samples of different kinds of cells. Due to a sophisticated design the columns allow very small elution volumes (5–50 µL) which results in highly concentrated DNA. Lysis is achieved by incubation of the sample material in a Proteinase K supplemented lysis buffer. Appropriate conditions for binding of the DNA to a silica membrane are created by adding ethanol. The mixture is applied to the column and DNA binds to a silica membrane. Washing steps efficiently remove contaminations and highly pure DNA is finally eluted with 5–50 µL of a elution buffer.

4 Reagents provided

The following components are included:

Code	Component	Quantity (# Tests)	Container
WB22	Pre-Lysis Buffer	50	Screw-cap bottle
WB23	Cell Lysis Buffer	50	Screw-cap bottle
WB19	DNA Wash Buffer	50	Screw-cap bottle
ES6	Proteinase K	50	Screw-cap bottle
WB20	Proteinase K Buffer	50	Screw-cap bottle (brown glas)
WB21	Elution Buffer*	50	Screw-cap bottle (yellow lid)
	Columns	50	Screw-cap bottle
	Collection Tubes (2ml)	50	
	Instructions for use	1	

*5 mM Tris/HCl, pH 8.5

5 Materials required but not provided

Reagents:

- Ethanol abs. p.A.
- H_2O (PCR-grade)

Equipment:

- 1.5 ml microcentrifuge tubes
- Pipettes
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Thermal heating-block (56°C)

6 Storage and handling

Store at 18...25°C in an upright position. If these storage conditions are followed, the kit will function, without loss of performance, at least until the expiry date printed on the label.

Upon storage, especially at low temperatures, a white precipitate may form in the Buffer solutions. Such precipitates can be easily dissolved by incubating the bottle at 50–70 °C before use.

After reconstitution the Proteinase K solution has to be stored at -16...-22°C (stable for 6 months).

Store diluted DNA Wash Buffer at room temperature (18...25°C) for up to one year.

Return to storage conditions immediately after use. Do not use reagents beyond expiration date indicated on the label. The device is stable until expiration date indicated on the label when handled accordingly.

7 Warnings and precautions

- Read the instructions for use prior to use!
- Do not use the reagents after the expiry date has been reached!
- A material safety data sheet is available on request for the professional user.
- Do not reuse reagents.
- Never pipet solutions with your mouth!

Hazards and precaution statements for Proteinase K (ES6):

Danger

H 315	Causes skin irritation.
H 319	Causes serious eye irritation.
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H 335	May cause respiratory irritation.
P 261	Avoid breathing dust.
P 280	Wear protective gloves / eye protection.
P 302+352	IF ON SKIN: Wash with plenty of water/…
P 304+340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P 305+351 +338	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.
P 312	Call a POISON CENTER/ doctor/ …/ if you feel unwell
P332+313	IF skin irritation occurs: Get medical advice/ attention
P 337+313	If eye irritation persists: Get medical advice/attention.
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
P403+233	Store in a well-ventilated place. Keep container tightly closed.

8 Limitations

- For Research use only.
- For professional use only.
- 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.
- DNA yield strongly depends on the sample type, quality, quantity, and time of storage.

9 Interfering substances

Not applicable.

10 Preparation of specimens

- Equilibrate cell sample to room temperature
- Resuspend cell sample and pipette 2 ml sample into a 2 ml microcentrifuge tube. If less than 2 ml cell sample is available, use the whole amount.

11 Preparatory treatment of the device

- **Proteinase K solution:** Add 1.35 ml **WB20** to dissolve 30 mg lyophilized **ES6**. Proteinase K solution is stable at -16...-22 °C for 6 months.
- **Diluted DNA Wash Buffer:** Add 48 ml 96–100 % ethanol to 12 ml **WB19**. Mark the label of the bottle to indicate that ethanol was added. Store diluted DNA Wash Buffer at room temperature (18...25 °C) for up to one year.
- Set incubator at 56°C.

12 Assay procedure

1. Lyse sample

   Centrifuge **5 min at 2,000 x *g*.** Discard supernatant, add 1ml H_2O (PCR-grade) and resuspend the pellet. Centrifuge for **5 min at 2,000 x *g*** and discard supernatant.

   Resuspend pellet in **200 µl WB22**, add **20 µl Proteinase K solution** and vortex vigorously.

   Add **200µl WB23**, vortex and incubate **10min at 56°C**. Spin down briefly.

2. Adjust binding conditions

   Add **200 µL ethanol abs. p. A.** to the tube and mix by **vortexing** (2 x 5 s). Spin down briefly to clear the lid.

   Avoid to centrifuge at much more then 1.000x g, because nucleic acid might precipitate. The ethanol will merge with the aqueous (lower) phase only.

3. Bind DNA

   For each sample, take one column placed in a CollectionTube (2 mL). **Apply the sample completely to the column**.

   Centrifuge for **1min at 20,000 x *g*.** Discard Collection Tube with flow-through and place the column in a new Collection Tube (2 mL).

4. Wash and dry silica membrane

   Add **500 µL diluted DNA Wash Buffer** to the column. Centrifuge for **1 min at 20,000 x *g***.

   Discard flow-through and place the column back into the Collection Tube.

   Add **500 µL diluted DNA Wash Buffer** to the column. Centrifuge for **1 min at 20,000 x *g***. Discard the Collection Tube with flow-through and place the column into new Collection Tube (2 mL).

   Centrifuge **3 min at 20,000 x g** to dry the membrane. Discard flow-through with Collection Tube.

5. Elute DNA

   Place the column in a new 1.5 ml microcentrifuge tube and apply **50 µl WB21** (5-50 µl) directly to the center of the silica membrane of the column. Incubate **5 min at room temperature** and centrifuge for **1 min at 20,000 x *g***.

13 Interpretation of results

Interpretations of results should be made according to the downstream assay protocol.

14 Recommended quality control procedures

Use standard DNA quality control procedures for determination of the DNA yield.

15 Performance characteristics

DNA yield strongly depends on the sample type, quality, quantity, and time of storage.

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 inferior results or to no results at all.

Observation	Possible cause	Recommended action
Low DNA yield	Low DNA content of the sample	The content of DNA depends on sample type, amount, and quality.
Column clogging	Sample contains residual cell debris or cells	The lysate may have contained residual particular matter. Make sure to proceed after the lysis step only with clear lysate.
No increase of PCR signal despite of an increased volume of eluate used as PCR template	Residual ethanol in eluate	Heat incubation of the elution fraction (incubate eluate with open lid for 8 min at 90 °C)
Discrepancy between A260 quantification values and PCR quantification values	Silica abrasion from the membrane	Due to the low DNA content in very small samples and the resulting low total amount of isolated DNA, a quantification via A260 absorption measurement is often hampered due to the low sensitivity of the absorption measurement. When performing absorption measurements close to the detection limit of the photometer, the measurement may be influenced by minor amounts of silica abrasion. In order to prevent incorrect A260 quantification of small DNA amounts centrifuge the eluate for 30 s at > 11,000 x *g* and take an aliquot for measurement without disturbing any sediment.
Unexpected A260 / A280 ratio	Measurement not in the range of photometer detection limit	In order to obtain a significant A260/A280 ratio it is necessary that the initially measured A260 and A280 values are significantly above the detection limit of the photometer used. An A280 value close to the background noise of the photometer will cause unexpected A260/A280 ratios

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

42 life sciences GmbH & Co. KG
Fischkai 1
27572 Bremerhaven/Germany
Phone: +49 471 4832-500
Fax: +49 471 4832-308
www.42ls.com
Email: info@42ls.com

Distributed by:
ZytoVision GmbH
Fischkai 1
27572 Bremerhaven/ Germany
Phone: +49 471 4832-300
Fax: +49 471 4832-509
www.zytovision.com
Email: info@zytovision.com