VisionArray
FFPE DNA Extraction Kit

REF VI-0001-50 Σ 50

For the DNA isolation from FFPE samples

For Research Use Only.
Not for use in diagnostic procedures

1 Intended use

The VisionArray FFPE DNA Extraction Kit (**VI-0001-50**) is intended to be used for the isolation of genomic DNA from formalin-fixed, paraffin-embedded (FFPE) specimens.

2 Clinical relevance

The use of FFPE samples for DNA analysis is limited due to chemical modification by formaldehyde and fragmentation of the DNA during tissue processing and storage of the samples. Standard DNA isolation procedures often result in low DNA yield or poor performance in downstream applications (e.g., PCR). A special purification system taking the unique requirements of FFPE tissue into account is inevitably necessary for successful analysis of nucleic acids from FFPE samples.

3 Test principal

The paraffin of FFPE sections is dissolved in the Paraffin Dissolver or xylene. The tissue is then digested by proteinase to solubilize the fixed tissue and release DNA into solution. Subsequently, heat incubation effectively eliminates crosslinks from the previously released DNA. After addition of ethanol, the lysate is applied to the column. DNA is bound to the silica membrane. Two washing steps help remove salts, metabolites, and macromolecular cellular components. Pure DNA is finally eluted in a small volume (20 µL) of Elution Buffer, yielding highly concentrated DNA.

4 Reagents provided

The following components are included:

Code	Component	Quantity (#Tests)	Container
WB16	Paraffin Dissolver	50	Screw-cap bottle
WB17	Tissue Lysis Buffer	50	Screw-cap bottle
WB18	Decrosslink Buffer	50	Screw-cap bottle
WB19	DNA Wash Buffer	50	Screw-cap bottle
ES6	Proteinase K	50	Screw-cap bottle (brown glas)
WB20	Proteinase K Buffer	50	Screw-cap bottle (yellow lid)
WB21	Elution Buffer*	50	Screw-cap bottle
	Columns	50	
	Collection Tubes (2ml)	50	
	Instructions for use	1	

*5 mM Tris/HCl, pH 8.5

5 Materials required but not provided

Reagents:

- 96–100 % ethanol (undenaturated ethanol is preferable)
- Optional for deparaffinisation without Paraffin Dissolver: Xylene, d-Limonene, mixtures of isoparaffinic hydrocarbons, or similar reagents for deparaffinization.

Equipment:

- 1.5 ml microcentrifuge tubes
- Pipettes
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Thermal heating-block (adjustable to 60 °C and 90 °C)

6 Storage and handling

Store at 18...25°C in an upright position. Storage at lower temperatures may cause precipitation of salts. 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 instruction 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!

Hazard and precautionary 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 isolated from FFPE tissue shows size distribution from 50 to 5,000 bases. Often short sized DNA from ca. 100–300 bases predominate, especially when the sample material is old.
- DNA yield strongly depends on the sample type, quality, quantity, and time of storage.
- The amount of paraffin is limited to 15 mg, when using the standard protocol with Paraffin Dissolver (approx. 7 sections of 10 µm x 250 mm^2). However, larger amounts of paraffin samples may be processed by using either additional Paraffin Dissolver or by deparaffinization using xylene. For samples comprising more than 15 mg paraffin, use 30 µL Paraffin Dissolver per 1 mg paraffin.

9 Interfering substances

Not applicable.

10 Preparation of specimens

Many factors influence the yield and usability of DNA obtained from FFPE samples. The procedure of tissue sampling, post sampling delay before fixation, fixation time, embedding, and storage conditions have a high impact on DNA quality and yield.
Starting from a paraffin embedded tissue block, samples should be sectioned under clean conditions. Paraffin sections may be stored at 2...8°C or lower for at least several weeks without observable effects on DNA yield or usability. Long term storage of paraffin sections may have a negative effect on the DNA due to air oxidation.

11 Preparatory treatment of the device

- **Proteinase K solution**: Add 1.35 ml of **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 **WB19**. Store diluted DNA Wash Buffer at room temperature (18...25°C) for up to one year.
- Set incubator(s) at 60°C and 90°C.
- Insert FFPE section(s) in microcentrifuge tube.

12 Assay procedure

12.1 DNA purification using Paraffin Dissolver

1. Deparaffinize sample

   Add **400 µl WB16** to the sample. Incubate **3 min at 60°C**. **Vortex** the sample immediately (at 60°C). Cool down sample to **room temperature**.

   Make sure that paraffin completely melts during the heat incubation step and mix well after melting to completely dissolve the paraffin.

2. Lyse sample

   Add **100 µl WB17**. **Vortex** vigorously. Centrifuge at **11,000 x *g* for 1 min.**

   Two phases will be formed: a lower (aqueous) phase and an upper (organic) phase. Tissue material will be transferred to the lower phase.

   Optional: The upper phase can be removed and discarded after centrifugation.

   Pipette **10 µl Proteinase K solution** directly into the lower phase. **Mix** the lower phase by pipetting up and down several times. (Pipette only the lower phase up and down. Avoid mixing lower phase and upper phase.)

   Make sure that the Proteinase K is mixed well with **WB17**.

   Incubate at **room temperature** for **3 hours** to lyse sample tissue.

   If residual unlysed tissue particles are visible after 3 hours, add additional 10 µL Proteinase K solution and continue digestion for further 3 hours or overnight.

   Yield of amplifiable DNA typically increases with elongated lysis time. During this incubation step protein is digested and DNA is released into solution.

   Vortex 5 s.

   Convenient stopping point: At this point, the procedure can temporarily be stopped. If pausing, we recommend to store the samples at -16…-22°C.

3. Decrosslink

   Add **100 µl WB18** to the tube and **vortex gently** to mix Buffer into the lower phase. Centrifuge at **11,000 x *g*** for **30 s** to obtain phase formation. Incubate at **90°C** for exactly **30 min**.

   Vortex 5 s and let cool down **to room temperature** (approx. 2 min).

   If necessary, spin down briefly to clear the lid.

4. Adjust binding conditions

   Add **200 µL ethanol** (96–100%) to the tube and mix by **vortexing** (2 x 5 s). **Spin down** briefly to achieve complete phase separation.

   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.

5. Bind DNA

   For each preparation, take one column placed in a CollectionTube (2 mL). **Pipette lower phase completely into the column**.

   It is recommended to pipette a volume of 450 µl on the column, to ensure that the complete lower phase is transferred. Small carry-over of the upper phase has no negative effect on the binding procedure.

   Centrifuge for **30 s** at **2,000 x *g*.** If the solution does not flow through completely, centrifuge for **30 s** at **11,000 x *g*** until the complete solution passed the column.

   Discard Collection Tube with flow-through and place the column in a new Collection Tube (2 mL).

6. Wash and dry silica membrane

Add **400 µL diluted DNA Wash Buffer** to the column. Centrifuge for **30 s** at **11,000 x *g***.

Discard Collection Tube with flow-through and place the column into a new Collection Tube (2 mL).

Add **400 µL diluted DNA Wash Buffer** to the column. Centrifuge for **2 min** at **11,000 x *g*** to dry the membrane. Discard the Collection Tube with flow-through and place the column into a new nuclease-free Collection Tube.

7. Elute DNA

Pipette **20 µL WB21** directly to the center of the silica membrane of the column.

Elution volume may be varied from 5–30 µL.

Centrifuge for **30 s at 11,000 x *g***.

8. Optional: Remove residual ethanol

*Incubate the eluate (20 µL) with open lid for **8 min** at **90°C**.*

12.2 DNA purification using xylene deparaffinization

1. Deparaffinize sample

Add **1 ml xylene** (or alternative reagent) to the sample. Incubate at room temperature until the paraffin is completely dissolved (approx. 2 min) and vortex vigorously (10 s).

Centrifuge for **2 min at 11,000 x *g***. Discard the supernatant by pipetting. Do not remove any of the pellet.

Add **1 ml ethanol (96–100 %)** to the pellet and vortex (5 s). Centrifuge for **2 min at 11,000 x *g***. Discard the supernatant by pipetting. Do not remove any of the pellet.

Incubate the open tube at **60°C** for **3–10 min** to dry the pellet.

It is important to evaporate all residual ethanol. Residual ethanol may reduce DNA yield.

2. Lyse sample

Add **100 µl WB17** and **10 µl Proteinase K solution** to the pellet. Vortex vigorously (5 s). Centrifuge briefly.

Solid section residuals at the tube wall should be flushed back into the solution by pipetting. Pipette solution up and down in order to homogenize sections.

Incubate at **room temperature** for **3 hours** to lyse sample tissue.

If residual unlysed tissue particles are visible after 3 hours incubation, add additional 10 µl Proteinase K solution and continue digestion for further 3 hours or overnight.

Yield of amplifiable DNA typically increases with elongated lysis time. During this incubation step protein is digested and DNA is released into solution.

Vortex tube 5 s.

Convenient stopping point: At this point, the procedure can temporarily be stopped. If pausing, we recommend to store the samples at -16...-22°C.

3. Decrosslink

Add **100 µl WB18** to the lysate and vortex vigorously (5 s).

Incubate at **90°C** for exactly **30 min**.

Vortex 5 s let cool down to room temperature (approx. 2 min. If necessary, spin down briefly to clear the lid.

4. Adjust binding conditions

Add **200 µl ethanol (96–100%)** to the lysate and **mix** by vortexing (2 x 5 s). Spin down briefly to clear the lid.

5. Bind DNA

For each preparation, take one column placed in a Collection Tube (2 mL).

Pipette lysate up and down two times before loading the lysate. Load the lysate into the column. Centrifuge for **30 s** at **2,000 x *g*.** If the solution does not flow through completely, centrifuge for **30 s** at **11,000 x *g*** until the complete solution passed the column.

Discard Collection Tube with flow-through and place the column in a new Collection Tube (2 mL).

6. Wash and dry silica membrane

Add **400 µl DNA Wash Buffer** to the column. Centrifuge for **30 s** at **11,000 x *g***.

Discard Collection Tube with flow-through and place the column into a new Collection Tube (2 mL).

Add **400 µl DNA Wash Buffer** to the column. Centrifuge for **2 min** at **11,000 x *g*** to dry the membrane. Discard the Collection Tube with flow-through and place the column into a new nuclease-free Collection Tube.

7. Elute DNA

Pipette **20 µl WB21** directly to the center of the silica membrane of the column.

Elution volume may be varied from 5–30 µl.

Centrifuge for **30 s** at **11,000 x *g***.

8. Optional: Remove residual ethanol

*Incubate the eluate (20 µl) with open lid for **8 min** at **90°C**.*

13 Interpretation of results

Due to tissue fixation, nucleic acids in FFPE samples are commonly fragmented and chemically modified by formaldehyde. Formaldehyde modifications of DNA cannot be detected by standard quality control assays, such as gel electrophoresis, spectrophotometry, fluorometry, or microfluidics analysis. However, efficiency of enzymatic reactions (e.g., PCR) with chemically modified DNA is significantly decreased.

14 Recommended quality control procedures

Predictions from DNA quality control procedures on the downstream PCR application should be made with care. The major quality indicator for DNA from FFPE samples is the performance in the intended application.

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
DNA is degraded/ no DNA obtained	Poor sample quality	Sample quality has a high impact on quality and amount of the DNA.
	Reagents not applied properly	Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added.
	Kit storage	Keep bottles tightly closed in order to prevent evaporation or contamination.
Poor DNA quality or yield	Ionic strength and pH influence A260 absorption as well as ratio A260/A280	For absorption measurement, use 5 mM Tris pH 8.5 as diluent.
Clogged column/ Poor DNA quality or yield	Too much starting material was used or Insufficient disruption and/or homogenization of starting material.	Reduce the quantity of sample material or use larger volumes of Paraffin Dissolver and/or Lysis Buffer FL.
		Use only supernatant for step 5 of chapter 12.2.
Suboptimal performance of DNA in downstream experiments	Carry-over of ethanol or salt	Do not let the flow-through touch the column outlet after the second wash with Buffer B5. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic Buffer B5 completely.
		Check if Buffer B5 has been equilibrated to room temperature before use. Washing at lower temperatures decreases efficiency of salt removal by Buffer B5.
		Depending on the robustness of the used PCR system, PCR might be inhibited if too much eluate is applied. Use less eluate as template.
	Store isolated DNA properly	Eluted DNA should always be kept on ice for optimal stability since possible traces of DNases will degrade the isolated DNA.
Discrepancy between A260 quantification values and PCR quantification values	Silica abrasion from the membrane	Due to the typically low DNA content in small FFPE samples and the resulting low total amount of isolated DNA, a DNA 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.
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