

# **Tissue DNA Isolation Kit**



**ARAMESH BI  GENE**

**Research, Development & production of  
Advanced Medical Diagnosis**

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**Designed for the isolation of DNA from Tissues and Formalin- fixed Paraffin Embedded Tissues (FFPET)**

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## Kit contents

Solution	Description	Storage	50 preps	100 preps
<b>ATL</b>	Tissue lysis Buffer	+16 to 25°C	12.5 ml	25 ml
<b>ADLB</b>	Lysis Buffer	+16 to 25°C	12.5 ml	25 ml
<b>AW1 (Ready to use)</b>	Inhibitor Removal Buffer	+16 to 25°C	25 ml	50 ml
<b>AW2 (Ready to use)</b>	Washing Buffer 2	+16 to 25°C	40 ml	80 ml
<b>ABB</b>	Binding Buffer	+16 to 25°C	9 ml	18 ml
<b>Proteinase K</b>	Sample lysis and inactivation of DNase	-20°C	1.25 ml	2.5 ml
<b>ADEB</b>	Elution Buffer	+16 to 25°C	5 ml	10 ml
<b>Spin column</b>	High pure silica membrane		50 pcs	100 pcs
<b>Collection tube</b>			150 preps	300 preps

**Storage Condition and Stability**

- 1- All solutions of ABG Tissue DNA Isolation Kit are clear and should be stored at Room Temperature (RT: +16 to +25°C).  
*! The buffers can show a slight yellow color. This will have no impact on the function of the buffer.*
- 2- When precipitates have formed in solutions, warm the solutions in 56°C water bath until the precipitate dissolve.
- 3- Store Proteinase K at -20°C. Repeated freezing and thawing Should be avoided.
- 4- All kit components are stable until the expiration date on the kit box, without showing any reduction in performance.
- 5- Improper storage at +2 to 8°C or -20°C will adversely impact nucleic acid purification because solutions might be precipitated.

**Additional Equipment (not provided)**

- 1- 1.5- or 2.0-mL micro-centrifuge tubes
- 2- Pipettes and filter tips (RNase free)
- 3- Standard tabletop microcentrifuge capable of 17,000 xg centrifugal force
- 4- Vortex mixer
- 5- Heat Block
- 6- Personal protection equipment (lab coat, gloves, goggles)

**For the isolation of nucleic acids from formalin-fixed paraffin-embedded tissue sections**

- Xylene
- Ethanol, 100%, 80%, 50%



## Product description

ABG Tissue DNA Isolation Kit is designed for rapidly and easily isolation of DNA from tissue samples, formalin-fixed paraffin- embedded tissues (FFPET).

This kit employs a proprietary lysis buffer in combination with spin column membrane to efficiently purify DNA from biological sample. The protocol provides a simple method to achieve rapid isolation of highly purified DNA. Isolated DNA has high quality metrics, including  $A_{260}/A_{280} > 1.8$  and  $A_{260}/A_{230} > 2.0$  and minimal residual RNA. DNA prepared by this kit is suitable for a variety of applications, such as PCR, southern blotting, RAPD, AFLP, RFLP and other molecular biology experiments.

## Sample Materials:

**25 to 50 mg mammalian tissue**

**Formalin-fixed paraffin embedded tissue**

## Quality Control

All components of ABG Tissue DNA Isolation Kit are manufactured in strictly clean conditions, and their degree of cleanliness is monitored periodically. To maintain consistency, a quality control process has been carried out thoroughly from lot to lot and only the approved qualified kit will be delivered. For quality control purpose, the DNA is isolated from cell lines and 250  $\mu$ l of EDTA whole blood previously by the manufacturer. Yield could be measured using spectrophotometry (OD) from samples.

## Warning and precautions

- 1- Wear disposable gloves, laboratory coats and eye protection when handling specimens as if potentially infectious and reagents.
- 2- Wash hand thoroughly after handling samples and reagents.
- 3- Use sterile, disposable plastic wares and filtered pipette tips.
- 4- Buffers of the kit contain irritants which are harmful when contact with skin and eyes, or when inhaled and swallowed. Avoid to contacting the lysis buffer and wash buffers with acidic solution and bleach.



- 5- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Areas. Do not return samples, equipment and reagents in the area where you performed in previous steps.
- 6- Safety Data Sheets (SDS) are available online.

### **Before you begin**

1. All centrifugation steps are carried out at room temperature (15 to 25°C).
2. Sample should be equilibrated to room temperature.
3. Check all reagent for any precipitation. If Lysis Buffer forms precipitate, please warm the it in a 56°C water bath until the precipitates dissolve.
4. Use fresh material to avoid degradation of genomic DNA.
5. Preheat thermo block or water bath to 70°C before starting the procedure.
6. Pre-fill the needed amount of ADEB into a sterile 2.0 ml reaction tube and incubate the ADEB at 75°C until the final step.



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# Hand Book

## Storage of samples

Sample type	Short term storage	Long-term storage
Tissue	2 weeks (+16 to +25°C)	-70°C
Tissues in VTM/ PBS/ Saline	48 hours (+2 to +8°C)	-70°C
formalin-fixed paraffin- embedded	(+16 to +25°C)	(+16 to +25°C)

**Procedure:****➤ Sample preparation:****❖ Formalin-fixed paraffin-embedded samples****Deparaffinization**

- 1- Add 1 ml xylene to five, 20  $\mu$ m thick section tissue.
- 2- Mix well by vortex and incubate for 5-15 minutes (depends on the thickness of the section).
- 3- Centrifuge for 3 minutes at 10,000 xg, and discard the supernatant.

*! Repeat this step depends on the thickness of the section.*

**Dehydration**

- |   |                 |
|---|-----------------|
| 4- Add 1 ml Ethanol respectively:<br><i>! After resuspending pellet in ethanol (100%,80%,50%), mix well by vortex, then centrifuge for 3 minutes at 10,000 xg, and discard supernatant.</i> | a. Ethanol 100% |
|   | b. Ethanol 80%  |
|   | c. Ethanol 50%  |

*! The section should turn to white color after it is adding the ethanol.*

**Rehydration**

- 5- Add 1 ml double-distilled water (DDW), mix well by vortex for 10 seconds.
- 6- Centrifuge for 3 minutes at 10,000 xg, and discard supernatant.
- 7- Incubate the tube for 20 minutes at 40°C.



## Tissue lysis step

8- To a nuclease free 1.5 ml microcentrifuge tube add:	a. Deparaffinized tissue
	b. 200 µl ATL.
	c. 25 µl P.K.

9- Mix immediately by pulse-vortex (20 seconds) and incubate at 56°C for 1 hour.

*! During the incubation, vortex the sample every 10 minutes.*

*! After this incubation, no tissue particles should be visible (depends on the thickness of tissue).*

*! If the tissue digested incompletely, add additional 20 µl P.K and incubate until the digestion is completed.*

10- Incubate for 1 hour at 90°C.

11- Proceed to protocol for DNA isolation.

### ❖ Tissue (stored at VTM/ PBS/ Saline)

## Tissue lysis step

1- To a nuclease free 1.5 ml microcentrifuge tube add:	a. Tissue (25- 50 mg)
	b. 250 µl ATL.
	c. 25 µl P.K.

2- Mix immediately by pulse-vortex (20 seconds) and incubate at 56°C for 1 hours.

*! During the incubation, vortex the sample every 10 minutes.*

*! After this incubation, no tissue particles should be visible (depends on the thickness of tissue).*



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**! If the tissue digested incompletely, add additional 20  $\mu$ l P.K and incubate until the digestion is completed.**

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3- Proceed to protocol for DNA isolation.

## ❖ Protocol for DNA Isolation

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### Before to begin

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- 1- Set a heating block to 70°C.
  - 2- Pre-fill the needed amount of ADEB into a sterile microcentrifuge tube and incubate the ADEB at 75°C until the elution step.
  - 3- Always mix the Proteinase K briefly before use.
- 

### DNA Lysing Step

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- |  |                |
|--|----------------|
| 1- To a nuclease free 1.5 ml microcentrifuge tube sample add:<br><i>(! Do not add Proteinase K directly to Binding buffer)</i> | a. 250 µl ADLB |
|--|----------------|
- 

- 2- Mix immediately by pulse-vortex (20 seconds) and incubate at 70°C for 10 minutes.  
***! During incubation, vortex the sample every 3-5 minutes.***
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### Binding Step

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- 3- Add 175 µl of ABB and mix well by pulse-vortex (20seconds).  
***! In the presence of undigested or precipitated remnants centrifuge at 10,000 rpm is recommended. Use supernatant for next step.***
  - 4- Assemble one spin column in to one collection tube.
  - 5- Pipette the liquid sample in to the upper reservoir of the spin column.
  - 6- Centrifuge for 30 seconds at 12,000 rpm.
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7- Remove the spin column from the collection tube and discard the flow through liquid, and the collection tube.

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8- Assemble the spin column with a new collection tube.

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## Washing Steps

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9- Add 500  $\mu$ l AW1 to the upper reservoir of the spin column.

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10- Centrifuge for 30 seconds at 12,000 rpm.

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11- Remove the spin column from the collection tube and discard the flow through liquid, and the collection tube.

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12- Assemble the spin column with a new collection tube.

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13- Add 600  $\mu$ l AW2 to the upper reservoir of the spin column.

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14- Centrifuge for 30 seconds at 12,000 rpm and discard the flow through.

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15- Add 200  $\mu$ l AW2 to the upper reservoir of the spin column.

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16- Centrifuge for 1 minute at 12,000 rpm and discard the flow through.

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17- Centrifuge for 3 minutes at 14,000 rpm to remove residual ethanol.

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18- Remove the spin column from the collection tube and discard the collection tube.

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## Elution Step

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19- Insert the spin column into a clean, sterile 1.5 ml microcentrifuge tube.

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20- Add 50-100  $\mu$ l of prewarmed ADEB to the upper reservoir of the spin column and incubate at RT (+16 to +25°C) for 2 minutes.



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21- Centrifuge for 1 minute at 12,000 rpm.

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22- Use the eluted DNA directly or store it at -20°C for short term use.

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23- For later analysis store eluted DNA at -70°C.



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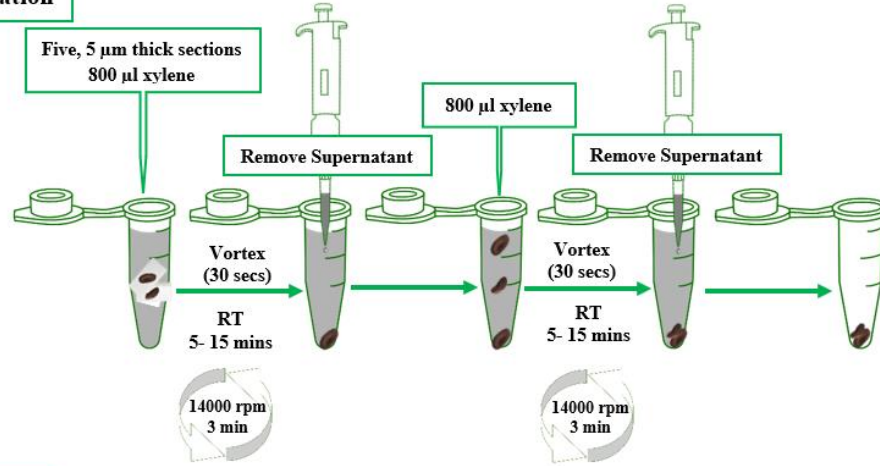
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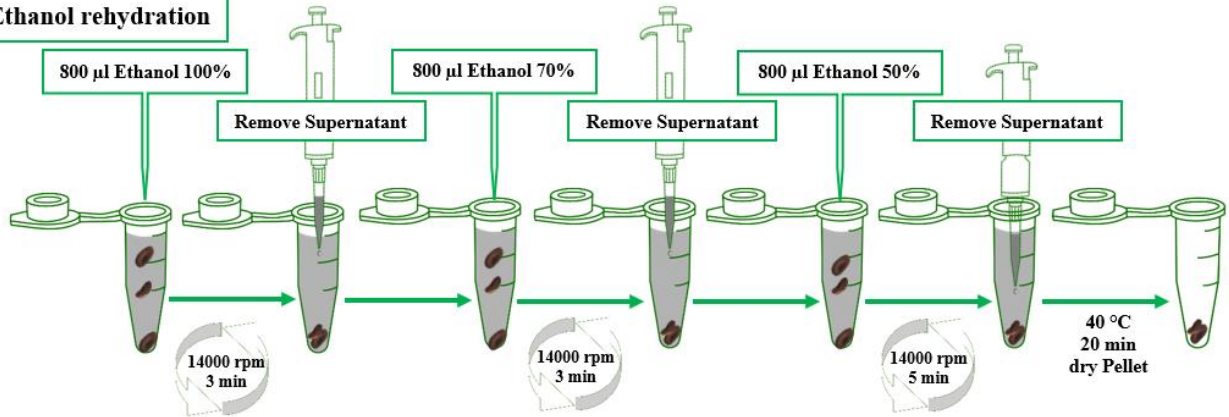
Date of last issue:

## Tissue DNA Isolation charts:

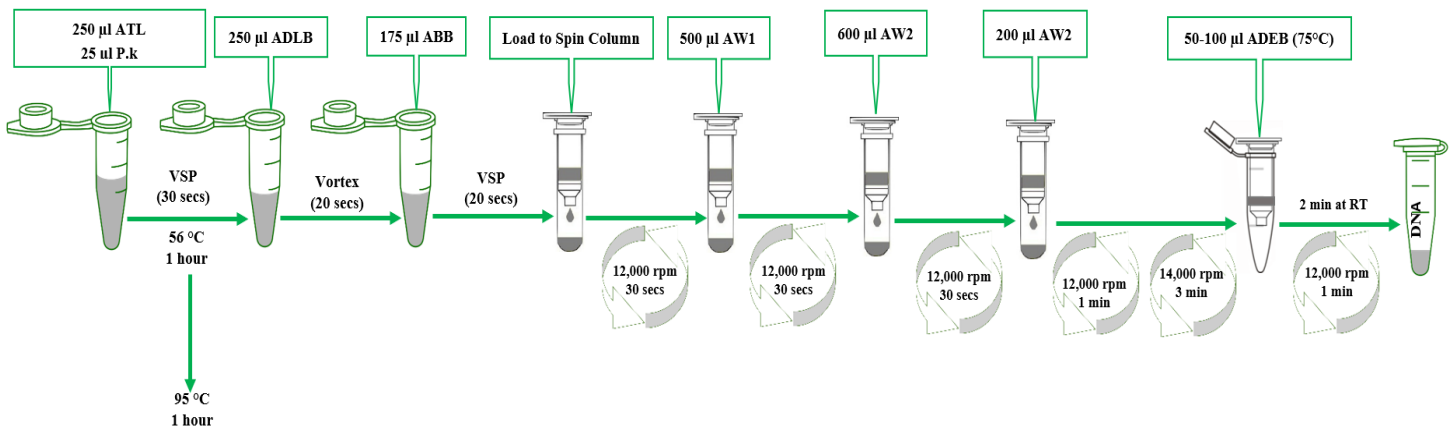
### Tissue deparaffinization



### Ethanol rehydration



### Tissue DNA Isolation





## Troubleshooting

This troubleshooting guide may be helpful for solving any problems that may arise. However, if you have questions or experience problems with this product. Please contact our Technical Support staff. Our scientists are committed to provide rapid and effective assistance.

Observation	Cause	Comment
<b>low nucleic acid concentration</b>	Kit stored under suboptimal conditions	Store kit contents according to the labeled temperature
	Buffers or other reagents were exposed to conditions that reduced their effectiveness	Store all buffers at +16 to +25 °C
		Close all reagents bottles tightly, to preserve pH, stability, ...
		Aliquot and store P.K at -20°C
	Incomplete cell lysis because of insufficient Proteinase K activity	Repeat the extraction procedure with a new sample. Use a fresh or well stored Proteinase K stock solution.
	Incomplete cell lysis because of insufficient mixing with ADLB	Repeat the extraction procedure with a new sample. Mix the sample and ADLB immediately and thoroughly by pulse-vortex (Recommended 45 seconds).
	Incomplete lysis because of insufficient incubation time	Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain.
	Clogged spin Filter (Inefficient disruption or homogenization)	<ul style="list-style-type: none"> <li>• Increase lysis time</li> <li>• Increase centrifugation</li> </ul> Reducing amount of starting material
Reagents and samples not completely mixed	Mix the sample tube completely after addition of each reagent.	

	Suboptimal reagent has been used for elution. Alkaline pH is required for optimal elution	Do not use water to elute nucleic acids from spin column. Use the elution buffer from the kit.
<b>Incompletely or no restriction enzyme cleavage product.</b>	Glass fibers which can co-elute with nucleic acid, scatter light	After elution step is finished, remove the spin column, and centrifuge the tube containing eluted sample for 1 minute at maximum speed. Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of original tube
<b>Absorbency (A<sub>260</sub>) reading of product is too high.</b>	Glass fibers which can co-elute with nucleic acid, scatter light	See suggestion under “Incompletely or no restriction enzyme cleavage product.” above.
<b>Low A<sub>260</sub>/280</b>	Eluate containing the purified DNA product is contaminated with ethanol from wash buffers.	After the last wash step, make certain flow through solution containing wash buffer does not contact the bottom of the high pure spin column. If this step has occurred, empty the collection tube and reinsert the contaminated filter, and re-centrifuge for 2 minutes.
<b>Purified DNA sample cannot easily be loaded into the well of an agarose gel, but instead “pops out” of the well as it is loaded.</b>	Eluate containing the purified DNA product is contaminated with ethanol from wash buffers.	See suggestion under “Low A <sub>260</sub> /280 (section a)” above.





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## Contact and Support

Aramesh Bio Gene appreciates its customers, and strives to make their experience the best it can be. Ask technical questions about all AB Gene products, from product choice, to product use. AB Gene support team composed of highly trained experienced scientists, who are able to troubleshoot most problems you may encounter.

Contact our technical support at any time by selecting one of these ways:

- phone: +9821- 22142231/22142883
- Email: [info@abiogene.ir](mailto:info@abiogene.ir)
- Company address: 1st floor, No. 11, Majd street, East Sarv Street, Kaj Square, Sa'adatabad, Tehran, Iran.