DNA AllGene Isolation Kit

ARAMESH BIO GENE

Research, Development & production of

Advanced Medical Diagnosis

2022

DNA AllGene Isolation Kit Hand Book

ARAMESH BIO GENE

Version: 1.0 Date of first issue: 10.27.2022 Date of last issue:

Designed for the isolation of Genomic, Viral, Bacteria, Yeast DNA from Blood, Buffy coat Cultured cell and Cell lines

Kit contents

Solution	Description	Storage	50 preps	100 preps
ATL	Tissue lysis Buffer	+16 to 25°C	10 ml	20 ml
ADLB	Lysis Buffer	+16 to 25°C	12.5 ml	25 ml
AW1 (Ready to use)	Inhibitor Removal Buffer	+16 to 25°C	25 ml	50 ml
AW2 (Ready to use)	Washing Buffer 2	+16 to 25°C	30 ml	60 ml
ABB	Binding Buffer	+16 to 25°C	9 ml	18 ml
Proteinase K	Sample lysis and inactivation of DNase	-20°C	1.25 ml	2.5 ml
G solution	DNA Binding	-20°C	500 µl	1 ml
ADEB	Elution Buffer	+16 to 25°C	5 ml	10 ml
Lysozyme	Bacteria DNA Isolation	+2 to +8°C	2 ml	4 ml
Spin column	High pure silica membrane		50 pcs	100 pcs
Collection tube			150 preps	300 preps

Storage Condition and Stability

1- All solutions of ABG DNA AllGene 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 and G solution 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%

For the isolation of nucleic acids from cell lines, whole blood, buffy coat or cultured cells

- PBS

For the isolation of nucleic acids from bacteria or yeast

- Lyticase (Yeast)
- PBS

Product description

ABG DNA AllGene Isolation Kit is designed for rapidly and easily isolation of DNA from a variety of sample sources including whole blood, buffy coat, serum, plasma, cultured cells, tissue samples and viruses. Bacteria & yeast require a specific pre-lysis treatment using lysozyme or lyticase.

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 A260/A280 > 1.8 and A260/A230 > 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:

200 to 300 µl mammalian whole blood (EDTA)

200 µl buffy coat

Serum

Plasma

Body fluid

25 to 50 mg mammalian tissue

Yeast cells

Bacterial cells

Formalin-fixed paraffin embedded tissue

Quality Control

All components of ABG DNA AllGene 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 µl 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 to 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.

Storage of samples

Sample type	Short term storage	Long-term storage
Whole blood (EDTA)	48 hours (+2 to +8°C)	-70°C
Serum	48 hours (+2 to +8°C)	-70°C
Plasma	48 hours (+2 to +8°C)	-70°C
Body fluids	48 hours (+2 to +8°C)	-70°C
Cell lines	48 hours (+2 to +8°C)	-70°C
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:

❖ Blood

1- If the sample volume is <250 µl, add the appropriate volume of sterile PBS.

2- If the sample volume is between 250 µl and 300 µl, increase all other sample volumes accordingly.

3- If RNA- free genomic DNA is required, add 4µl of 100mg/ml RNase A solution (optional).

❖ Cultured cells, amniotic fluid, papilloma specimens (thin prep, oral cells, urine), cell lines, ...

1- Transfer up to 1-3 ml of specimens to a centrifuge tube.

2- Centrifuge for 5 min at 10,000 xg, discard the supernatant.

! For Isolation from Amniotic Fluid (AF) or poor cell samples, store 250 µl of supernatant together with the pellet and proceed to protocol for DNA isolation.

3- Resuspend the cell pellet in 250 µl PBS.

4- Proceed to protocol for DNA isolation.

❖ Formalin-fixed paraffin-embedded samples

Deparaffinization

1- Add 1 ml xylene to five, 20 µm 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: ! *After resuspending pellet in ethanol (100%,80%,50%), mix well by vortex, then centrifuge for 3 minutes at 10,000 xg, and discard supernatant*.	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. 200 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).

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

3- Proceed to protocol for DNA isolation.

❖ Bacteria/ Yeast

Specimens

Serum/plasma (not recommended)	**Buffy coat**	**CSF**
Sputum: decontaminated in NALC-NaOH (See specimen decontamination procedure)	**Bone marrow**	**Urine**

Nasopharyngeal and Oropharyngeal swab

! Place the swab directly into a vial containing 180 µl lysozyme, and vortex for 30 seconds.

! If the swab preserved in PBS, proceed to bacteria sample preparation section.

Sample preparation

1- Centrifuge sample for 10 minutes at 17,000 xg, and discard the supernatant.

2- Resuspend cell pellet in:	Bacteria: 180 µl lysozyme (10 mg/ml). Yeast: 180 µl lyticase (10 mg/ml)
3- Mix immediately by pulse-vortex for 30 seconds.	
4- Incubate at 37°C:	Bacteria: for 30 minutes ! MTB: for 1 hour Yeast: for 1 hour
5- Add 250 µl working solution.	

Working solution preparation

6- To a 1.5 ml nuclease free microcentrifuge tube add:	a. 250 µl ADLB b. 10 µl G solution c. Internal Control (Refer to instruction of Real time PCR kit).
7- Incubate at 56°C for 30 minutes.	
8- Incubate at 95°C for 15 minutes. *! Samples no longer infectious, only after completion of the incubation at 95°C.* *! The risk of aerosol- mediated contamination after opening the tube is extremely high.*	
9- Cool down the sample to room temperature (+16 to +25°C).	
10- Proceed to protocol for DNA isolation from step No. 3.	

Specimen decontamination procedure

NaOH - sodium citrate working solution

1- Prepare an appropriate volume of NaOH-sodium citrate working solution according to below table.

Preparation of 2% NaOH-1.45% Sodium Citrate Working Solution

Volume needed (ml)	NaOH (grams)	Sodium Citrate x 2 H_2O (grams)
250	5	3.125
500	10	7.25
1000	20	14.5
2000	40	29

2- Add the correct amount of NaOH and sodium citrate together into two thirds of the required volume of distilled water in a conical flask and dissolve completely.

3- Once dissolved completely, make up to the appropriate volume with distilled water.

4- Dispense 250ml volumes into screw cap Duran bottles. Attach autoclave tape on each bottle, label, date and autoclave them for 15 minutes at 121°C, 15 psi. Allow to cool.

5- Store at room temperature for up to 4 weeks.

0.5% NALC-2% NaOH-1.45% sodium citrate solution

1- Pre-weight 0.25g amounts of N-Acetyl-L-cysteine into labeled sterile screw cap bottles and store in the refrigerator. Sufficient bottles for approximately 1 month can be prepared at one time.

2- Immediately prior to use, transfer 50ml of NaOH-sodium citrate solution into a sterile graduated conical tube in aseptical condition.

3- Aseptically, add 0.25g NALC into the solution and allow to dissolve completely before use.

4- The solution should be fresh and prepared immediately before use.

0.067 M Phosphate Buffer, pH 6

1- Dissolve Na2HPO4 and KH2PO4 in distilled water according to table. Check pH on pH-meter or with pH strips.

Total Volume (ml)	Disodium phosphate (Na_2HPO_4) anhydrous (grams)	Monopotassium phosphate (KH_2PO_4) (grams)
1000	4.74	4.54
2000	9.47	9.07
3000	14.20	13.60

! If final buffer requires pH adjustment, add Na_2HPO_4 powder to raise the pH of the solution or KH_2PO_4 to lower it.

1- Distribute the buffer into 500ml volumes in screw capped Duran bottles. Label, date and attach autoclave tape over the lid.

2- Autoclave at 121°C, 15 psi. for 15 minutes. Allow to cool, store at room temperature. Unopened bottles can be stored up to 4 weeks.

Specimen Processing

1- Label centrifuge tubes with specimen numbers and place in a rack.

2- Open only one specimen and one tube at a time. Transfer up to 10 ml of specimen into 50 ml sterile disposable conical tube. For very small amount or for viscous specimens (like sputum), add a small volume of phosphate buffer to the specimen container and mix the contents to loosen the specimen and to facilitate sputum transfer.

3- Add equal amount of fresh NALC-NaOH-sodium citrate working solution to each tube, opening only one tube at a time. Addition of equal amount of NALC-NaOH-sodium citrate working solution is critical.

4- Recap the tube tightly and agitate on a vortex mixer for no more than 30 seconds.

! Avoid excessive agitation, as it may inactivate NALC and cause the sample to coagulate.

5- Let the tube remains for 15 minutes at room temperature to decontaminate the specimen.

6- Make sure the specimen is completely liquefied. If still mucoid, add small amount of NALC powder (30 – 35 mg) directly to the specimen tube. Mix well by inverting the tube several times.

7- Processing time can be extended for up to 20 – 25 minutes, but longer time not recommended.

8- Add sterile phosphate buffer, pH 6.8 up to the 45 ml mark. This will reduce the continued action of the NaOH and lower the viscosity of the mixture. Recap the tubes tightly and mix well by inverting several times.

9- Using aerosol-free sealed centrifuge cups, centrifuge the specimen tubes at 3,000*g* for 20 minutes.

***!** Open the aerosol-free sealed centrifuge cups ONLY inside the biological safety cabinet. Remove the centrifuge tubes.*

10- Opening one tube at a time, carefully with one uninterrupted movement to decant the supernatant into the splash-proof discard container containing approximately 5cm depth of suitable disinfectant. Make sure the sediment is not lost during decanting. Recap the tube.

11- Opening one tube at a time, draw up an appropriate volume of phosphate buffer, pH 6.8 into a sterile disposable Pasteur pipette (usually 1-2ml, depending on the number of tests being performed). Add the buffer along the wall of the tube holding the end of the pipette close to the sediment to prevent aerosolization. Mix thoroughly with the pipette. Discard the pipette into sharp container.

12- Recap the tube tightly and proceed to **bacteria/yeast sample preparation.**

❖ Protocol for DNA Isolation

Before to begin

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

1- To a nuclease free 1.5 ml microcentrifuge tube add: *(! Do not add Proteinase K directly to Binding buffer)*	a. 250 µl sample b. 250 µl ADLB c. 25 µl P. K d. 10 µl G solution ***! in case of <u>Viral/ Amniotic fluids</u> or poor pellet samples.***

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.

Binding Step

3- Add 175 µl of ABB and mix well by pulse-vortex (20seconds).

! In the presence of undigested or precipitated remnants (cell lines) 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.

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

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

Washing Steps

9- Add 500 µl AW1 to the upper reservoir of the spin column.

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

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

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

13- Add 600 µl AW2 to the upper reservoir of the spin column.

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

15- Add 200 µl AW2 to the upper reservoir of the spin column.

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

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

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

Elution Step

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

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

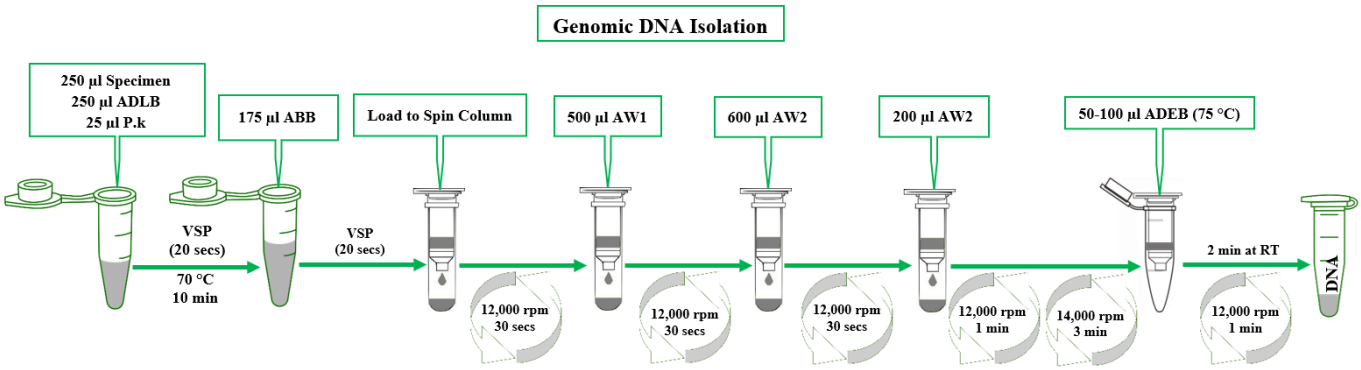
21- Centrifuge for 1 minute at 12,000 rpm.

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

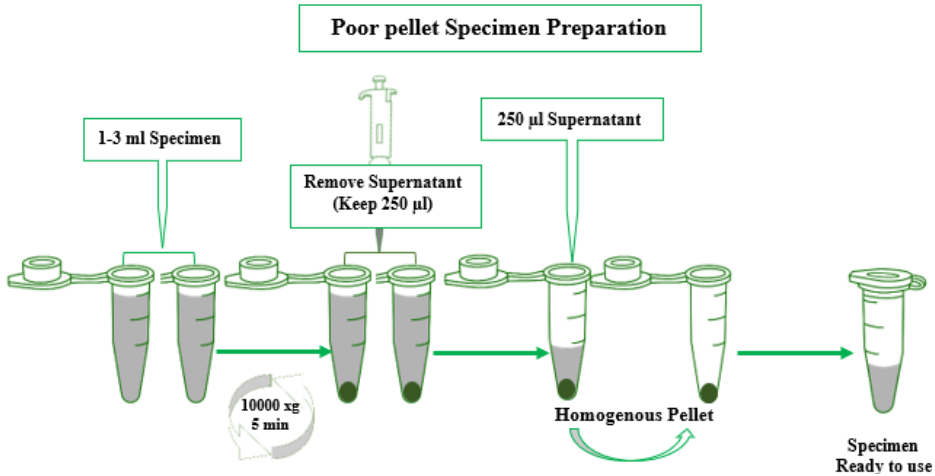
23- For later analysis store eluted DNA at -70°C.

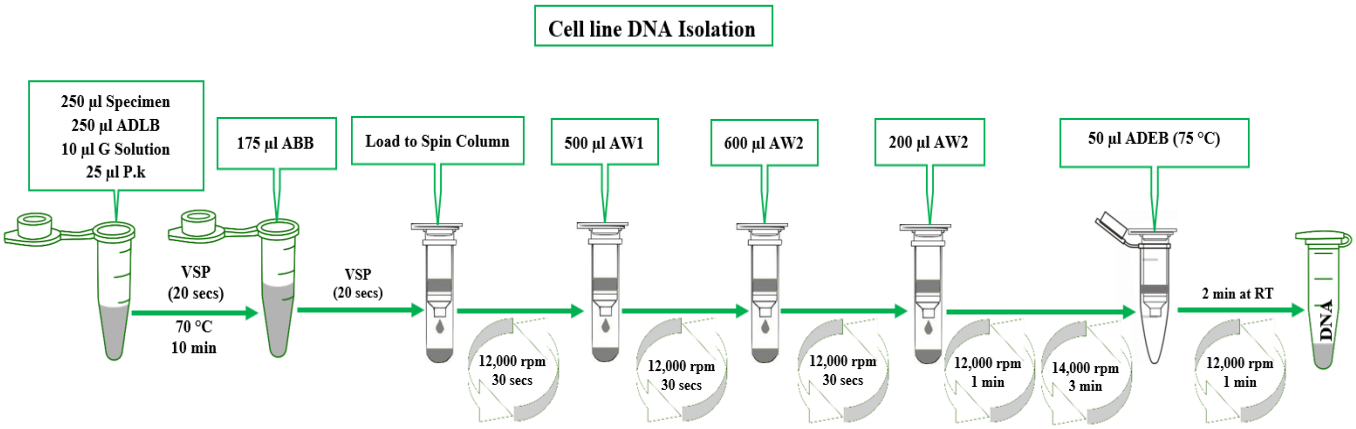
DNA Isolation charts:

Genomic DNA Isolation:



Cell lines: Poor pellet Isolation (Amniotic fluid, Urine, thin prep, oral swab, …)

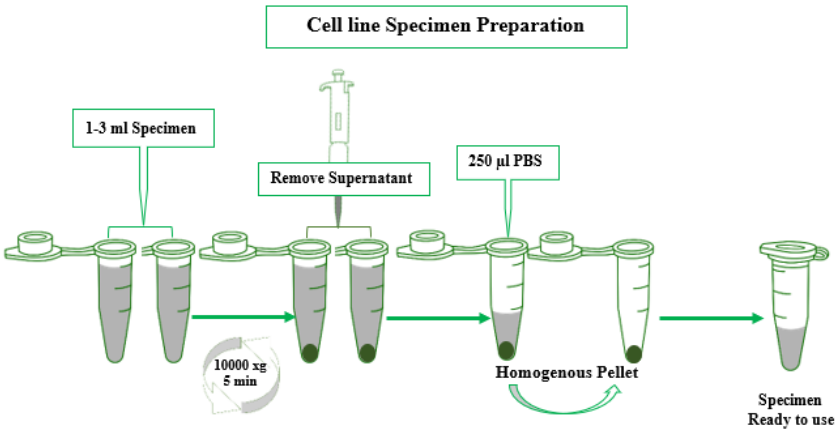


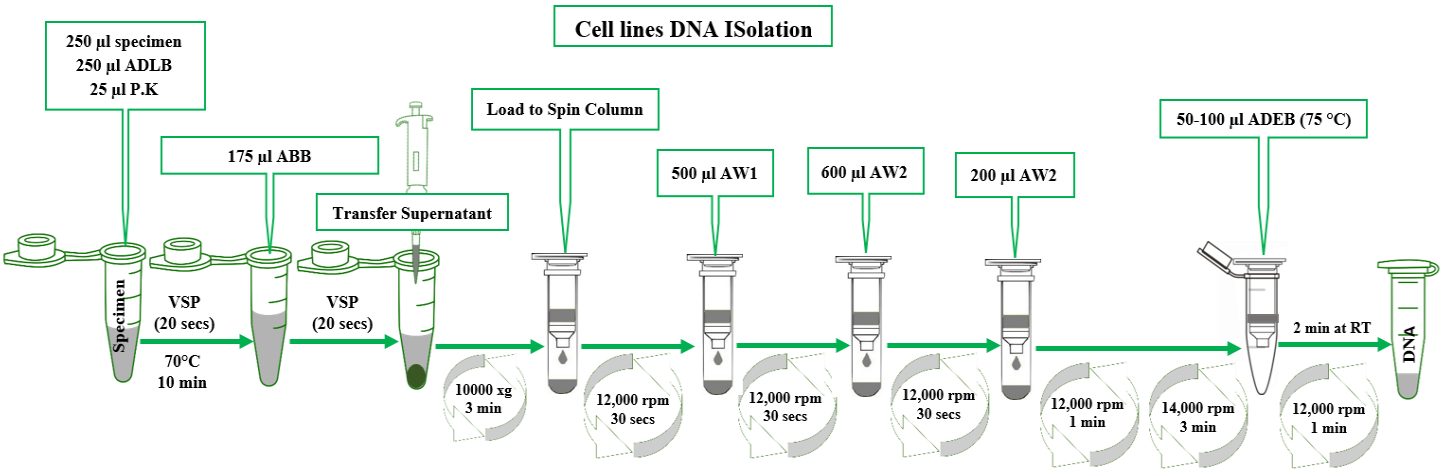


DNA AllGene Isolation Kit Hand Book

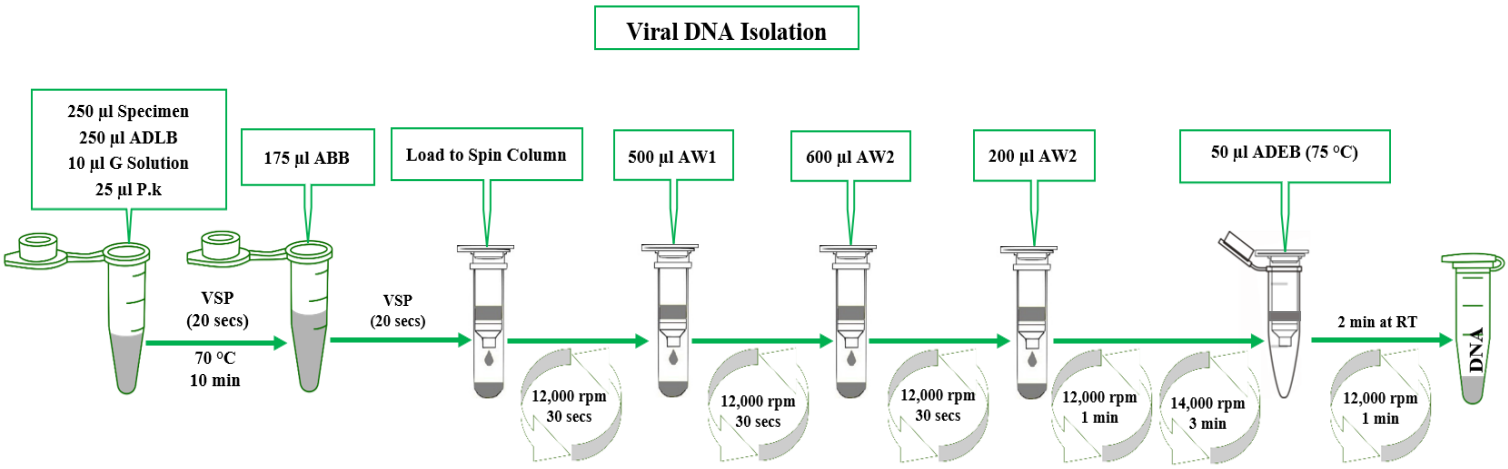
Version: 1.0 Date of first issue: 10.27.2022 Date of last issue:

Cell lines: thin prep, cell culture, oral swabs, …

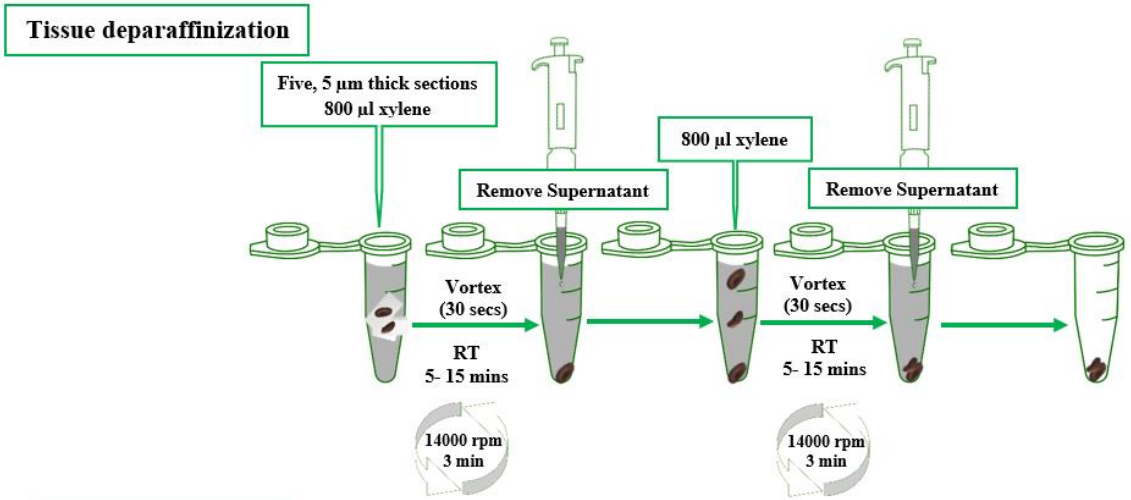


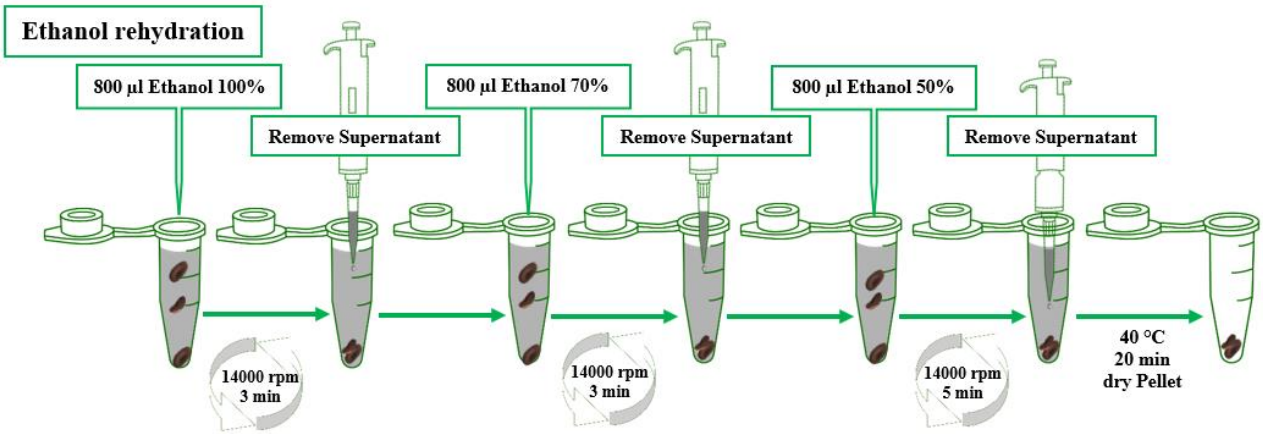


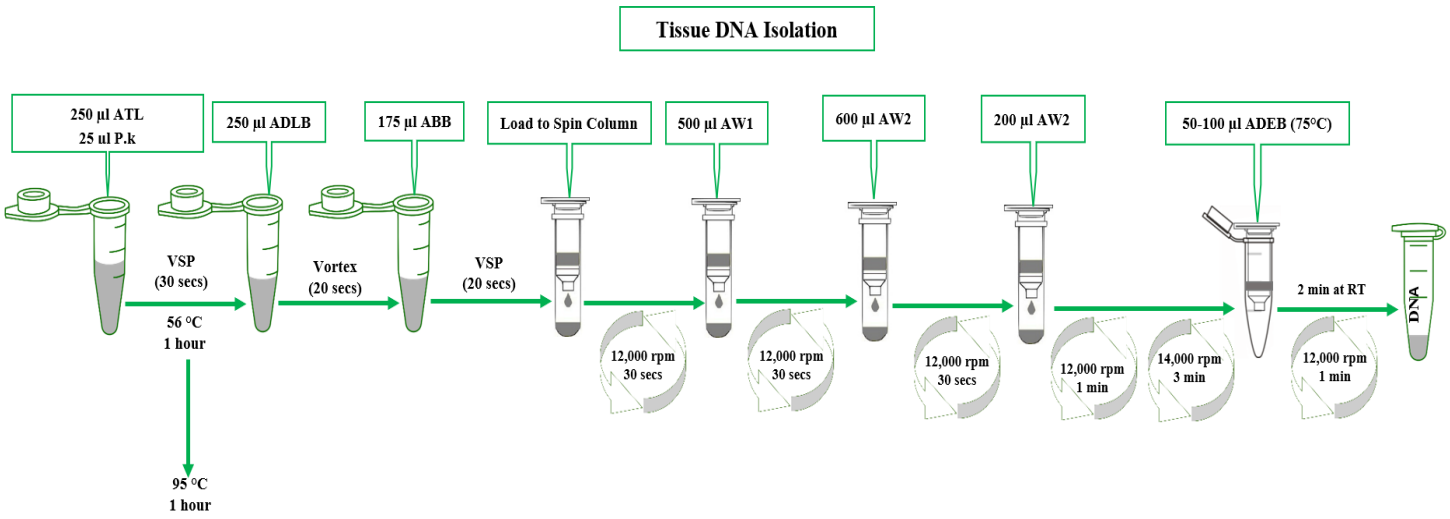
Viral DNA Isolation (serum, plasma, body fluids,…)



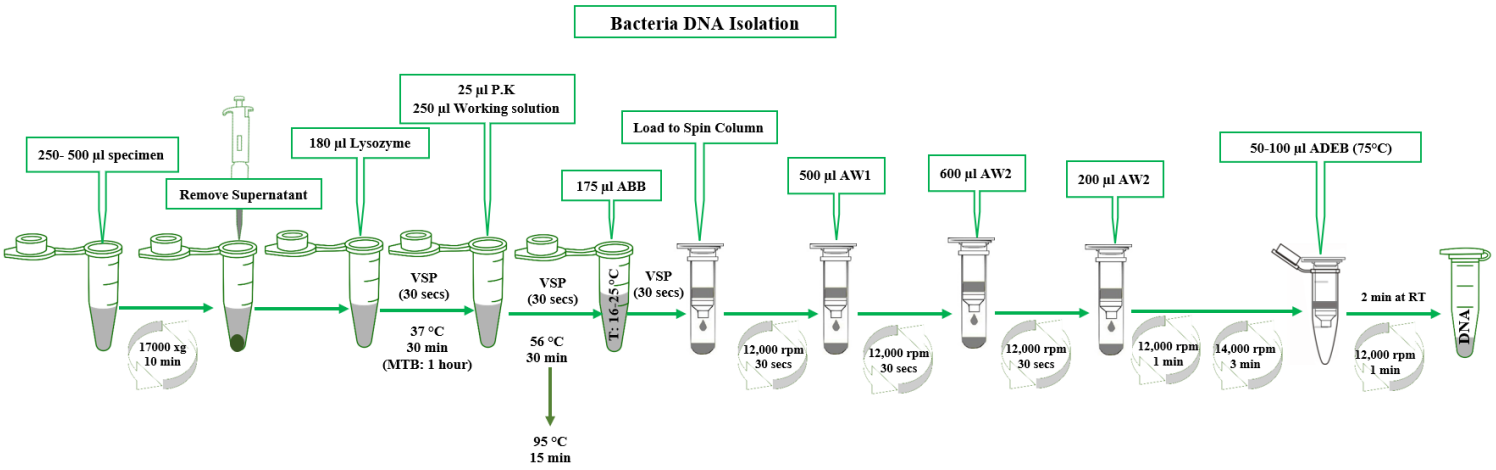
Tissue DNA Isolation:







Bacteria 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
low nucleic acid concentration	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 ℃
		Close all reagents bottles tightly, to preserve pH, stability, …
		Aliquot and store P.K, G solution, lysozyme 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)	• Increase lysis time • Increase centrifugation 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.
	Smaller amounts of sample material used than specified.	Use 5µl G solution as DNA binder.
Incompletely or no restriction enzyme cleavage product.	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
Absorbency (A_{260}) reading of product is too high.	Glass fibers which can co-elute with nucleic acid, scatter light	See suggestion under "Incompletely or no restriction enzyme cleavage product." above.
Low A260/280	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.
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.	Eluate containing the purified DNA product is contaminated with ethanol from wash buffers.	See suggestion under "Low A260/280 (section a)" above.

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
- Company address: 1st floor, No. 11, Majd street, East Sarv Street, Kaj Square, Sa'adat Abad, Tehran, Iran.