# Bacterial DNA Isolation Kit


**Research, Development & production of**

**Advanced Medical Diagnosis**

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# Bacteria DNA Isolation Kit Hand Book

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

**Designed for the isolation of Bacteria DNA from various type of samples**

## Kit contents

| Solution | Description | Storage | 50 preps | 100 preps |
|---|---|---|---|---|
| **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 | 40 ml | 80 ml |
| **ABB** | Binding Buffer | +16 to 25°C | 9 ml | 17.5 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 | -20°C | 9 ml | 18 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 Bacteria 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 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)

## Product description

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

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.

## Sample Materials:

**200 to 300 µl mammalian whole blood (EDTA)**

**200 µl buffy coat**

**Serum**

**Plasma**

**Body fluid**

**Bacterial cells**

**Sputum**

## Quality Control

All components of ABG Bacteria 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 sputum 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 |

## Procedure:

### Bacteria DNA Isolation sample preparation:

| | | |
|---|---|---|
| **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.*

### ❖ Serum, plasma, whole blood, Cultured cells, (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 poor cell samples, store 250 µl of supernatant together with the pellet and proceed to protocol for DNA isolation.***

3- Proceed to protocol for DNA isolation.

### 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 Lysis

1- Transfer between 250 µl and 500 µl of the NALC-NaOH-decontaminated sample into a 1.5 ml screw-cap tube (sputum samples)

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

| | |
|---|---|
| 3- Resuspend cell pellet in: | Bacteria: 180 µl lysozyme (10 mg/ml). |

4- Mix immediately by pulse-vortex for 30 seconds.

| | |
|---|---|
| 5- Incubate at 37°C: | Bacteria: for 30 minutes<br>! MTB: for 1 hour |

6- Add 250 µl working solution.

### Working solution preparation

| | |
|---|---|
| 7- To a 1.5 ml nuclease free microcentrifuge tube add: | a. 250 µl ADLB |
| | b. 10 µl G solution |
| | c. Internal Control<br>(Refer to instruction of Real time PCR kit). |

8- Add 25 µl of PK and mix well by pulse-vortex (20seconds).

9- Incubate at 56°C for 30 minutes.

10- 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.***

11- Cool down the sample to room temperature (+16 to +25°C).

## Binding Step

1- 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.***

2- Assemble one spin column in to one collection tube.

3- Pipette the liquid sample in to the upper reservoir of the spin column.

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

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

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

## Washing Steps

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

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

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

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

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

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

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

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

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

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

## Elution Step

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

18- 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.

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

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

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

# Bacteria DNA Isolation Kit Hand Book

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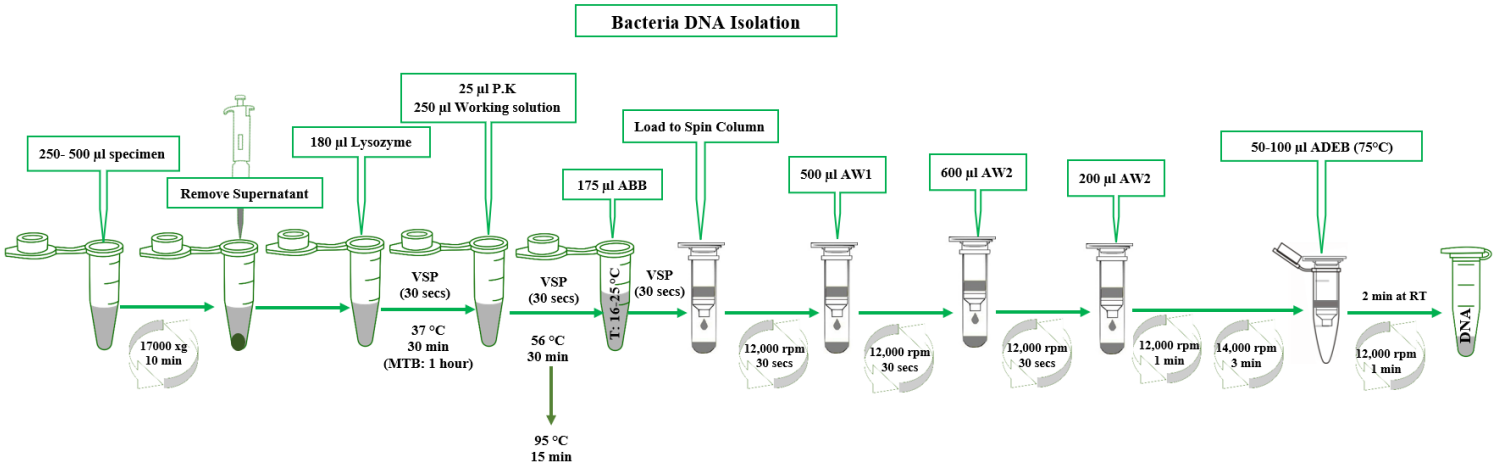
## Bacteria DNA Isolation charts:



## 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<br>• Increase centrifugation<br>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.<br>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'adatabad, Tehran, Iran.