ARPENspin Omni Genomic DNA Extraction Kit

Kit Components

Cat#	ARP-GDES50
COMPONENTS	
TES Buffer	14mL
TET Buffer	9mL
LysisB Buffer	25mL
PK Solution	1mL
RNase A Solution	200µL
PW Buffer	12mLa
Wash Buffer	24mLb
Elution Buffer	5mL
Spin Columns	50

Storage & shipping conditions

1. Please store Protease K and RNase A at a temperature between 2 and 8 degrees Celsius. Store all other components at room temperature.
2. All components remain stable for 18 months under proper storage conditions from the date of receipt.

Introduction

The kit is a fast, simple, and cost-effective way to isolate high-quality DNA. It uses a single protocol to extract purified DNA from various samples such as animal tissue, whole blood, buffy coat, leukocytes, bacteria, yeast, blood spots, swabs, and cultured cells. The kit employs silica-based membrane techniques in a convenient spin column, eliminating the need for expensive resins, hazardous phenol-chloroform extractions, or time-consuming alcohol precipitation. The entire process, including lysis steps, takes less than 20 minutes. This kit can purify more than 20 kb DNA. The kit can purify DNA fragments of more than 20 kb, suitable for direct application to PCR, Southern blotting, and other enzymatic reactions.

Typical genomic DNA yields from various samples

Source	Quantity	Yield (µg)
Mammalian tissue	25mg	5-30
Mammalian blood	200µL	2-10
Bacterium	$\leq 10^9$	≤ 50
Cell	$\leq 2\times10^6$	5-30
Blood spots	2-5 pieces	0.1-1

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Additional apparatus and materials required but not supplied

* Sterile 1.5mL microcentrifuge tubes
* Centrifuge capable of 12,000g
* Vortex mixer
* PBS buffer
* Lyticase
* 10μL/200μL/1000μL tips
* Absolute ethanol
* Warm bath
* Lysozyme
* Sorbitol buffer (18.217g sorbitol + 3.7224g EDTA-2Na, dissolve into 100 mL ddH_2O)

Important Notes

Please be sure to add absolute ethanol to the PW buffer and Wash buffer, and mix thoroughly before using.

Protocol

- **Sample lysis**
  - **Cell and blood**

1. Sample pre-processing

   A. **Culture cells**: adherent cells shall be digested with trypsin, no more than 10^7cells. Spin the tube at 1500rpm for 5min, discard the supernatant, add 200μL of PBS buffer and re-suspend the cells.
   B. **Leukocytes**: if blood volume is more than 200μL, use Red Blood Cell Lysis Buffer first to obtain white blood cells, add 200μL PBSbuffer into the tube, re-suspend cells.
   C. **Whole blood**: Add 200μl of well-mixed blood to the tube. If the blood volume is less than 200μl, add PBS buffer to make it 200μl.
2. Add 20μL PK solution, mix thoroughly for 15s. Add 4μL RNase A. Incubate at room temperature for 2min.
3. Add 200μL Lysis B buffer and mix thoroughly. Incubate at 56℃ for 10min.
4. Add 200μL absolute ethanol and mix thoroughly.

  - **Animal tissue**

1. Add 180μL TES Buffer and 20μL PK solution into tube.
2. Grind tissue to powder (no more than 25mg) with liquid nitrogen. Add it to the tube and mix thoroughly.
   Note: for the spleen, no more than 10mg samples must be submerged within the solution.
3. Incubate for 1-4 hours at 56℃. For samples complex to lysis, prolong lysis time or evenovernight.
   Note: vortex per 10s will facilitate lysis.
4. Centrifuge 3min at 12,000g. Carefully transfer the supernatant into a new tube.
5. Add 4μL RNase A and incubate at room temperature for 2min.
6. Add 200μL Lysis B buffer and mix thoroughly.
7. Add 200μL absolute ethanol and mix thoroughly.

  - **Blood spots**

1. Take 2-5 pieces of spots (3×3mm) into a tube.
2. Add 280μL TES Buffer and 20μL of PK solution into the tube. Mix intensively for 10s. Put it in 56℃ thermostat oscillator metal bath, vortex for 60min at 900rpm.
3. Centrifuge for 3min at 12,000g. Carefully transfer the supernatant into a new tube.
4. Add 4μL RNase A and incubate at room temperature for 2min.
5. Add 300μL Lysis B buffer and mix thoroughly.
6. Add 150μL absolute ethanol and mix thoroughly.

  - **Gram negative bacteria lysis**

3. Harvest up to 2 x 10^9 bacterium cells in a microcentrifuge tube by centrifuging for 1 minute at maximum speed. Dispose of as much supernatant as possible.
4. Resuspend the pellet in 180 μL of TES buffer and 20 μL of PK solution. Mix by pulse-vortexing intensively for 20 seconds.
5. Incubate at 56℃ for 30 minutes-4hours.

Optional: add 4μL RNase A (20mg/mL) and mix thoroughly

1. Add 200μL LysisB buffer, and mix thoroughly.
2. Add 200μL absolute ethanol, mix thoroughly.

■ **Gram positive bacteria lysis**

1. Please add Lysozyme to TET Buffer to a final concentration of 20 mg/mL. Mix thoroughly until the solution is clear. Store at 2-8°C.
2. Harvest a maximum of 2 x 10^9 bacterium cells by centrifuging in a microcentrifuge tube for 1 minute at maximum speed. Discard the supernatant as much as possible.
3. Resuspend the pellet in 180 μL TES Buffer. Mix by pulse-vortexing intensively for20 seconds.
4. Incubate at 37℃ for 30 minutes.
5. Add 20μL PK solution and mix thoroughly.
6. Add 200μL LysisB buffer and mix thoroughly. Incubate at 55℃ for 30 minutes
7. Add 200μL absolute ethanol and mix thoroughly.

■ **Yeast culture lysis**

1. Resuspend the pellet of yeast cells in 500μL of sorbitol buffer. Add 100 to 200 units of lyticase and incubate the mixture at 37°C for 30 minutes. Centrifuge for 1 minute at 6,000 x g and discard the supernatant.
2. Add 180μL TES Buffer and 20μL PK solution，vortex the tube for 20sec.
3. Incubate at 56℃ for 30 minutes.
4. Optional: add 4μL RNase A (20mg/mL) and mix thoroughly. Incubate at room temperature for 2 minutes.
5. Add 200μL LysisB buffer and mix thoroughly.
6. Add 200μL absolute ethanol and mix thoroughly.

■ **Swab**

1. Sample preparation： Use cotton swabs in cheek wipes 10-15 times.
2. Turn the swab into a 2 mL centrifuge tube; cut out the stem part from the swab with scissors.
3. Add 500μL LysisB Buffer and 20μL PK Solution to the 2.0mL microcentrifuge tube. Mix thoroughly for 10 seconds.
4. Incubate at 56℃ for 15 minutes.
5. Take 350μL the lysis product to the 1.5mL microcentrifuge tube.
6. Add 350μL of ethanol and mix thoroughly for10 seconds.

➢ **DNA Purification**

1. Transfer the mixture (≤750μL) to a spin column and centrifuge at 12,000×g for 1 minute. Discard the flow-through.
2. Add 500μL PW buffer to the Spin column. Centrifuge the spin column at 12,000×g for 1 minute. Discard flow-through.
3. Add 500μL Wash buffer to the spin column—centrifuge at 12,000 ×g for 1 minute. Discard flow-through. Take the spin column back to the tube.
4. Repeat step 3
5. Centrifuge the spin column at 12,000 ×g for 2 minutes.
6. Place the spin column in a new 1.5 or 2.0mL microcentrifuge tube. Add 30-100μL of the Elution Buffer. Incubate at room temperature for 2 minutes. Centrifuge the mixture at 10,000×g for 1 minute. The DNA in the collection tube is ready for further analysis. If the isolated DNA sample is not tested on the same day, freeze it at -20℃.