ARPENspin Omni Genomic DNA Extraction Kit

Cat# ARP-GDES50

Kit components

COMPONENT	QUANTITY
TES Buffer	14 mL
TET Buffer	9 mL
LysisB Buffer	25 mL
PK Solution	1 mL
RNase A Solution	200 µL
PW Buffer	12 mL (add 18 mL absolute ethanol)
Wash Buffer	24 mL (add 36 mL absolute ethanol)
Elution Buffer	5 mL
Spin Columns	50

Storage and transport

Transport the kit at room temperature. Store PK Solution (Protease K) and RNase A Solution at 2–8°C. Store all other components at 2–30°C. Shelf life is 18 months under these conditions. Observe the expiry date on the box.

Principle

The kit is designed for rapid, simple, cost-effective isolation of high-quality DNA from animal tissue, whole blood, buffy coat, leukocytes, bacteria, yeast, blood spots, swabs and cultured cells. After sample-specific lysis, all samples follow the same silica spin-column purification procedure. No expensive resins, phenol–chloroform extraction or alcohol precipitation are required. DNA fragments longer than 20 kb can be used directly for PCR, Southern blotting and other enzymatic reactions.

Typical genomic DNA yields

SAMPLE	INPUT	YIELD (µg)
Mammalian tissue	25 mg	5–30
Mammalian blood	200 µL	2–10
Bacteria	≤10^9 cells	≤50
Cells	≤2 × 10^6 cells	5–30
Blood spots	2–5 pieces	0.1–1

Before you begin

Prepare sterile 1.5 mL microcentrifuge tubes, 10 µL, 200 µL and 1000 µL tips, a centrifuge suitable for the speeds and forces specified below, a vortex mixer, a warm bath, absolute ethanol and PBS. Use sterile DNase-free tubes and tips. Do not mix kit components from different lots.

Wash buffers. Before first use, add 18 mL absolute ethanol to the 12 mL PW Buffer and 36 mL absolute ethanol to the 24 mL Wash Buffer. Mix each buffer thoroughly.

Additional materials include lysozyme for Gram-positive bacteria and lyticase for yeast. For sorbitol buffer, dissolve 18.217 g sorbitol and 3.7224 g disodium EDTA (EDTA-2Na) in 100 mL of double-distilled water.

Cells and blood

Prepare the starting sample

Cultured cells. For adherent cells, digest with trypsin. Use no more than 10^7 cells. Centrifuge at 1,500 rpm for 5 minutes, discard the supernatant and resuspend the cells in 200 µL PBS.

Leukocytes. If the starting blood volume exceeds 200 µL, first use Red Blood Cell Lysis Buffer to obtain leukocytes. Resuspend the leukocytes in 200 µL PBS.

Whole blood. Use 200 µL of well-mixed blood. If using less than 200 µL, add PBS to bring the total volume to 200 µL.

Lyse the prepared cells or blood

1. Add 20 µL PK Solution and mix thoroughly for 15 seconds. Add 4 µL RNase A, mix and incubate at room temperature for 2 minutes.
2. Add 200 µL LysisB Buffer, mix thoroughly and incubate at 56°C for 10 minutes.
3. Add 200 µL absolute ethanol and mix thoroughly. Proceed to DNA purification on page 4.

Animal tissue

1. Add 180 µL TES Buffer and 20 µL PK Solution to a tube. Finely chop up to 25 mg of tissue or grind it in liquid nitrogen. Add the tissue to the tube and mix thoroughly. For spleen, use no more than 10 mg and keep the sample submerged.
2. Incubate at 56°C for 1–4 hours. For tissue that is difficult to lyse, extend the incubation, up to overnight. During incubation, vortex for 10 seconds at regular intervals to aid digestion.
3. Centrifuge at 12,000 rpm for 3 minutes. Carefully transfer the supernatant to a new tube. Add 4 µL RNase A, mix and incubate at room temperature for 2 minutes.
4. Add 200 µL LysisB Buffer and mix thoroughly. Add 200 µL absolute ethanol, mix thoroughly and proceed to page 4.

Blood spots

1. Place 2–5 blood spot pieces, each 3 × 3 mm, in a tube. Add 280 µL TES Buffer and 20 µL PK Solution. Mix vigorously for 10 seconds.
2. Vortex in a temperature-controlled shaking metal bath at 56°C and 900 rpm for 60 minutes. Centrifuge at 12,000 rpm for 3 minutes. Carefully transfer the supernatant to a new tube.
3. Add 4 µL RNase A and incubate at room temperature for 2 minutes. Add 300 µL LysisB Buffer and mix thoroughly. Add 150 µL absolute ethanol, mix thoroughly and proceed to page 4.

Gram-negative bacteria

1. Collect up to 2 × 10^9 bacterial cells in a microcentrifuge tube by centrifuging at maximum speed for 1 minute. Remove as much supernatant as possible.
2. Resuspend the pellet in 180 µL TES Buffer and 20 µL PK Solution. Pulse-vortex vigorously for 20 seconds. Incubate at 56°C for 30 minutes to 4 hours. Vortex for 10 seconds at regular intervals to aid digestion.
3. Optional: Add 4 µL RNase A (20 mg/mL) and mix thoroughly.
4. Add 200 µL LysisB Buffer and mix thoroughly. Add 200 µL absolute ethanol, mix thoroughly and proceed to page 4.

Gram-positive bacteria

1. Dissolve lysozyme in TET Buffer to a final concentration of 20 mg/mL. Mix thoroughly for about 30 seconds until the solution is clear. Store at 2–8°C.
2. Collect up to 2 × 10^9 bacterial cells in a microcentrifuge tube by centrifuging at maximum speed for 1 minute. Remove as much supernatant as possible.
3. Resuspend the pellet in 180 µL TES Buffer. Pulse-vortex vigorously for 20 seconds, then incubate at 37°C for 30 minutes.
4. Add 20 µL PK Solution, mix thoroughly and incubate at 56°C for 30 minutes. Add 200 µL absolute ethanol, mix thoroughly and proceed to page 4.

Yeast

1. Resuspend the yeast pellet in 500 µL sorbitol buffer. Add 100–200 units of lyticase and incubate at 37°C for 30 minutes. Centrifuge at 6,000 × g for 1 minute and discard the supernatant.
2. Add 180 µL TES Buffer and 20 µL PK Solution. Vortex for 20 seconds and incubate at 56°C for 30 minutes.
3. Optional: Add 4 µL RNase A (20 mg/mL), mix thoroughly and incubate at room temperature for 2 minutes.
4. Add 200 µL LysisB Buffer and mix thoroughly. Add 200 µL absolute ethanol and mix thoroughly.

Cheek swabs

1. Do not eat or drink for 30 minutes before sampling. Use the toothed part of the swab to rub the inner cheek between the upper and lower teeth more than 20 times, moving back and forth and up and down with brushing pressure. Avoid inflamed areas. Repeat on the other cheek with the same swab. Place the swab in a 2 mL centrifuge tube and cut off the stem with scissors.
2. Add 500 µL LysisB Buffer and 20 µL PK Solution. Mix thoroughly for 10 seconds and incubate at 56°C for 15 minutes.
3. Transfer 350 µL of lysate to a 1.5 mL microcentrifuge tube. Add 350 µL ethanol and mix thoroughly for 10 seconds.

DNA purification

1. Transfer up to 750 µL of the prepared mixture to a spin column. Centrifuge at 12,000 rpm for 1 minute and discard the flow-through.
2. Add 500 µL PW Buffer to the column. Centrifuge at 12,000 rpm for 1 minute and discard the flow-through.
3. Add 500 µL Wash Buffer. Centrifuge at 12,000 rpm for 1 minute, discard the flow-through and return the column to the tube.
4. Repeat step 3. Then centrifuge the column at 12,000 rpm for 2 minutes.
5. Place the column in a new 1.5 mL or 2.0 mL microcentrifuge tube. Add 30–100 µL Elution Buffer and incubate at room temperature for 2 minutes. Centrifuge at 10,000 rpm for 1 minute.
6. The purified DNA in the collection tube is ready for analysis. If the DNA will not be tested the same day, freeze it at −20°C.

Contact details

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