Spin Universal DNA Kit

Cat.No.: HYC221

Product Description

This kit enables high-purity total DNA extraction from a wide range of samples, including fresh/frozen animal tissues, cultured cells, whole blood and bacteria. It yields DNA fragments up to 50 kb in size. The purification workflow eliminates toxic organic solvents such as phenol and chloroform, and requires no ethanol precipitation step. The kit utilizes an optimized buffer system, which facilitates efficient and specific binding of genomic DNA to the silica membrane spin column upon sample lysis. Two sequential wash steps thoroughly remove PCR inhibitors and other enzymatic reaction contaminants. Purified high-quality DNA is eluted with low-salt buffer or nuclease-free water. Eluted DNA is ready for downstream applications including restriction digestion, standard PCR, quantitative real-time PCR, library construction, Southern blot hybridization and molecular marker analysis.

Components

Components	50T	200T
Buffer GTL	15 mL	60 mL
Buffer GL	15 mL	50 mL
Buffer GW1*	13 mL	52 mL
Buffer GW2*	15 mL	70 mL
Buffer GE	15 mL	60 mL
Proteinase K	1.25 mL	4×1.25 mL
Spin Columns DM with Collection Tubes	50	200

Storage

All components can be stably stored in a dry place at room temperature (15-30℃).

Required Reagents and Equipment

- Absolute Ethanol
- Enzymatic Lysis Buffer (required for genomic DNA extraction of Gram-positive bacteria) Composition of Enzymatic Lysis Buffer: 20 mM Tris, pH 8.0, 2 mM Na_2-EDTA, 1.2% Triton X-100, Lysozyme at a final concentration of 20 mg/mL

Instructions

Preparations

- Avoid repeated freeze-thaw cycles of samples, which will result in shorter DNA fragments and reduced DNA yield.
- For bacterial cultures with abundant secondary metabolites or thick cell walls, harvest samples at the early logarithmic growth phase for genomic DNA extraction.
- Prior to first use, add absolute ethanol to Buffer GW1 and Buffer GW2 as instructed on the reagent bottle labels.
- Check Buffer GTL and Buffer GL for crystals or precipitates before use. If any form, fully redissolve the buffers by incubation in a 56 °C water bath.
- If downstream assays are highly susceptible to RNA contamination, add 4 µL DNase-Free RNase A (100 mg/mL) before adding Buffer GL. RNase A is not supplied with this kit and can be purchased separately from our company upon request.

Genomic DNA Extraction from Blood and Cell Samples

1. Sample Pretreatment

1) For mammalian anticoagulated blood (enucleated red blood cells): Directly add Buffer GTL to 50–200 µL fresh or frozen anticoagulated blood to make up a final volume of 200 µL.
2) For anticoagulated blood from avians, amphibians and lower organisms (nucleated red blood cells): Take 5–10 µL fresh or frozen anticoagulated blood, then top up to 200 µL with Buffer GTL.
3) For adherent cultured cells: Prepare cell suspension first (maximum input: 5×10^6 cells). Centrifuge at 2,000 rpm (400 × g) for 5 min, discard all supernatant, add 200 µL Buffer GTL, and vortex thoroughly to fully resuspend the cell pellet.

Note: To eliminate RNA contamination, add 4 µL RNase A (100 mg/mL) after the above steps, vortex for 15 sec, and incubate at room temperature for 2 min.

2. Add 20 µL Proteinase K.
3. Add 200 µL Buffer GL, vortex vigorously to mix well, then incubate in a 56 °C water bath for 10 min.
4. Briefly centrifuge to spin down liquid droplets on the inner lid of the tube. Add 200 µL absolute ethanol, vortex thoroughly to homogenize, then perform a short spin-down.
   Note: Vortex immediately after adding Buffer GL and absolute ethanol. A white precipitate may form after adding Buffer GL and absolute ethanol, which does not interfere with downstream experiments. Some tissues may turn into a sol-like mixture; vigorous vortexing is recommended in this case.
5. Transfer all lysate from the previous step into Spin Column DM pre-assembled with a collection tube. If the total volume exceeds the column capacity, load in multiple aliquots. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min. Discard flow-through and reinsert the spin column into the collection tube.
6. Add 500 µL Buffer GW1 (confirm absolute ethanol has been added before use) to the spin column. Centrifuge at 12,000 rpm for 1 min, discard flow-through, and replace the spin column back into the collection tube.
7. Add 500 µL Buffer GW2 (confirm absolute ethanol has been added before use) to the spin column. Centrifuge at 12,000 rpm for 1 min, discard flow-through, and re-install the spin column on the collection tube.
   Note: Repeat Step 7 for higher DNA purity if required.
8. Centrifuge at 12,000 rpm for 2 min and discard all flow-through. Leave the spin column at room temperature for several minutes to fully air-dry the silica membrane.
   Note: This step removes residual ethanol from the spin column. Ethanol carryover will inhibit downstream enzymatic reactions (restriction digestion, PCR, etc.).
9. Place the spin column into a new self-supplied microcentrifuge tube. Pipette 50–200 µL of Buffer GE or sterile water onto the center of the silica membrane without touching the membrane surface. Incubate at room temperature for 2–5 min, then centrifuge at 12,000 rpm for 1 min to collect purified DNA. Store DNA at −20 °C.
   Notes:

1) **Use sterile water for elution if downstream assays are sensitive to pH or EDTA. Elution efficiency is highly dependent on eluate pH. If sterile water is used, adjust its pH to 7.0–8.5 with NaOH; elution yield will drop significantly at pH below 7.0.**
2) **Pre-warm Buffer GE in a 65–70 °C water bath and extend room-temperature incubation to 5 min prior to centrifugation to boost DNA yield. A second elution with another 50–200 µL Buffer GE or sterile water can further increase total DNA recovery.**
3) **To obtain higher DNA concentration, reload the eluate back onto the spin column, incubate at room temperature for 2–5 min, and centrifuge at 12,000 rpm for 1 min. A smaller elution volume (<200 µL) yields more concentrated DNA but reduces total DNA output. An elution volume of 50 µL Buffer GE or sterile water is recommended when expected DNA yield is less than 1 µg.**
4) **DNA stored in water undergoes acid hydrolysis over time. For long-term storage, elute DNA with Buffer GE and store at −20 °C.**

Genomic DNA Extraction from Animal Tissues

1. Sample Pretreatment

For animal tissue samples, use 25 mg tissue (spleen tissue input should be less than 10 mg). For rodent tails: take one 0.4–0.6 cm rat tail segment, or two 0.4–0.6 cm mouse tail segments.

1) Grind tissue with liquid nitrogen or cut into small pieces and transfer to a 1.5 mL microcentrifuge tube. Add 180 µL Buffer GTL and label each sample clearly.
2) If homogenizing with a tissue homogenizer: add no more than 80 µL Buffer GTL to the sample before homogenization, then supplement with another 100 µL Buffer GTL after homogenizing.

   Note:Do not exceed the recommended tissue input weight for all sample types. Pre-grinding tissues in liquid nitrogen or homogenizing in Buffer GTL improves lysis efficiency.

2. Add 20 µL Proteinase K and vortex thoroughly to fully homogenize the sample. Incubate in a 56 °C water bath until complete tissue lysis. Invert or vortex the tube periodically during incubation to disperse tissue clumps.

Notes:

1) **Digestion duration varies by tissue type; complete lysis generally takes 1–3 hours. Rodent tails require 6–8 hours digestion, and overnight incubation is acceptable with no adverse impact on downstream procedures.**
2) **If gelatinous material remains after incubation and vortexing, extend incubation at 56 °C or add an additional 20 µL Proteinase K for further digestion.**
3) **To remove RNA contamination, add 4 µL RNase A (100 mg/mL) after the above digestion step, vortex for 15 sec, and incubate at room temperature for 5–10 min.**

3. Add 200 µL Buffer GL and vortex vigorously for full mixing. Incubate in a 70 °C water bath for 10 min. Perform a brief spin-down, add 200 µL absolute ethanol, then vortex thoroughly to homogenize.

   Notes:

   1) **Vortex immediately after adding Buffer GL and absolute ethanol.**
   2) **A white precipitate may form upon addition of Buffer GL and absolute ethanol, which does not interfere with downstream experiments. Certain tissues (e.g., spleen, lung) may form a sol-like mixture; vigorous vortexing is recommended in such cases.**

4. Briefly centrifuge the tube, then transfer all lysate from Step 3 into Spin Column DM pre-installed on a collection tube. Load the lysate in multiple aliquots if the volume exceeds column capacity. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min, discard the flow-through, and reinsert the spin column into the collection tube.
5. Add 500 µL Buffer GW1 (confirm absolute ethanol has been supplemented before use) to the spin column. Centrifuge at 12,000 rpm for 1 min, discard flow-through, and place the spin column back into the collection tube.
6. Add 500 µL Buffer GW2 (confirm absolute ethanol has been supplemented before use) to the spin column. Centrifuge at 12,000 rpm for 1 min, discard flow-through, and re-install the spin column on the collection tube.

   Note: Repeat Step 6 to further elevate DNA purity if needed.

7. Centrifuge at 12,000 rpm for 2 min and discard all flow-through. Place the spin column at room temperature for several minutes to fully air-dry the silica membrane.

Note: This step eliminates residual ethanol inside the spin column. Ethanol carryover inhibits downstream enzymatic reactions (restriction digestion, PCR, etc.).

8. Transfer the spin column to a new self-supplied microcentrifuge tube. Pipette 50–200 µL Buffer GE or sterile water onto the center of the silica membrane without touching the membrane surface. Incubate at room temperature for 2–5 min, then centrifuge at 12,000 rpm for 1 min to collect purified DNA. Store DNA at −20 °C.

   Notes

   1) Use sterile water for elution if downstream assays are sensitive to pH or EDTA. Elution efficiency is strongly affected by eluate pH. If sterile water is used as eluent, adjust its pH to 7.0–8.5 with NaOH; elution recovery will decrease sharply at pH below 7.0.

   2) Pre-warm Buffer GE in a 65–70 °C water bath and extend room-temperature incubation to 5 min prior to centrifugation to increase DNA yield. A second elution with another 50–200 µL Buffer GE or sterile water can further boost total DNA recovery.

   3) To obtain higher DNA concentration, reload the collected eluate back onto the spin column, incubate at room temperature for 2–5 min, and centrifuge at 12,000 rpm for 1 min. An elution volume smaller than 200 µL generates more concentrated DNA but reduces total recovery. An elution volume of 50 µL Buffer GE or sterile water is recommended when expected DNA output is less than 1 µg.

   4) DNA stored in pure water is susceptible to acid hydrolysis. For long-term storage, elute DNA with Buffer GE and preserve at −20 °C.

Genomic DNA Extraction from Bacteria

1. Pre-treatment of Bacterial Samples

- **Gram-Negative Bacteria**

1) Transfer 1–5 mL bacterial culture (10^6–10^8 cells, maximum input ≤ 2×10^9 cells) into a self-supplied microcentrifuge tube. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min, and remove supernatant completely.

2) Resuspend the cell pellet in 180 µL Buffer GTL by vortexing.

3) Add 20 µL Proteinase K and mix thoroughly by vortexing. Incubate at 56 °C until full bacterial lysis. Invert or vortex the tube periodically during incubation to disperse cell clumps.

Note: To eliminate RNA contamination, add 4 µL RNase A (100 mg/mL) after the above lysis step, mix well and incubate at room temperature for 5–10 min.

4) Add 200 µL Buffer GL and vortex to homogenize thoroughly.

- **Gram-Positive Bacteria**

1) Transfer 1–5 mL bacterial culture (10^6–10^8 cells, maximum input ≤ 2×10^9 cells) into a self-supplied microcentrifuge tube. Centrifuge at 12,000 rpm for 1 min, and aspirate all supernatant thoroughly.
2) Resuspend the pellet in 180 µL self-prepared Enzymatic Lysis Buffer.
3) Incubate at 37 °C for 30 min.
4) Add 20 µL Proteinase K and vortex vigorously to mix evenly. Add 200 µL Buffer GL and vortex thoroughly, then incubate at 56 °C for 30 min.

Notes:

1) If pathogen inactivation is required, incubate the sample at 95 °C for 15 min; however, this high-temperature incubation may trigger partial DNA degradation.

2) To eliminate RNA contamination, add 4 µL RNase A (100 mg/mL) upon completion of the above steps, mix well and incubate at room temperature for 5–10 min.

2. Add 200 µL absolute ethanol and vortex vigorously for full homogenization.

Note: A white precipitate may form after ethanol addition, which will not interfere with downstream experiments.

3. Load the entire mixture from Step 2 (including any precipitate) into Spin Column DM pre-assembled with a collection tube. Load in multiple portions if the volume exceeds column capacity. Centrifuge at 12,000 rpm for 1 min, discard flow-through, and reinsert the spin column back into the collection tube.
4. Add 500 µL Buffer GW1 (verify absolute ethanol has been added before use) to the spin column. Centrifuge at 12,000 rpm for 1 min, discard flow-through, and reinstall the spin column onto the collection tube.

5. Add 500 µL Buffer GW2 (verify absolute ethanol has been added before use) to the spin column. Centrifuge at 12,000 rpm for 1 min, discard flow-through, and reinsert the spin column into the collection tube.

   Note: Repeat Step 5 to achieve higher DNA purity if necessary.

6. Centrifuge at 12,000 rpm for 2 min and discard all flow-through. Place the spin column at room temperature for several minutes to fully air-dry the silica membrane.

   Note: This step removes residual ethanol retained on the column membrane. Ethanol carryover will suppress downstream enzymatic reactions, such as restriction digestion and PCR.

7. Transfer the spin column to a new self-supplied microcentrifuge tube. Pipette 50–200 µL Buffer GE or sterile water onto the center of the silica membrane without contacting the membrane surface. Incubate at room temperature for 2–5 min, then centrifuge at 12,000 rpm for 1 min to collect purified DNA. Store DNA at −20 °C.

   Notes:

   1) Use sterile water for elution if downstream applications are sensitive to pH or EDTA. Elution efficiency is strongly dependent on the pH of the eluent. When sterile water is used, adjust its pH to 7.0–8.5 with NaOH; elution recovery will decrease significantly at pH below 7.0.

   2) Pre-warm Buffer GE in a 65–70 °C water bath and extend room-temperature incubation to 5 min prior to centrifugation to improve DNA yield. A second elution with an additional 50–200 µL Buffer GE or sterile water can further increase total DNA recovery.

   3) For higher final DNA concentration, reload the collected eluate onto the spin column, incubate at room temperature for 2–5 min, and centrifuge at 12,000 rpm for 1 min. Elution volumes smaller than 200 µL produce more concentrated DNA but reduce total yield. An elution volume of 50 µL Buffer GE or sterile water is recommended when expected DNA mass is less than 1 µg.

   4) DNA suspended in water is prone to acid hydrolysis during storage. For long-term preservation, elute DNA with Buffer GE and store at −20 °C.