Cat.NO. GERC-101076-100

Store the kit at 15 to 25℃

Genorise Universal DNA Mini Kit 100

For purification of total DNA from

animal blood

animal tissue

rodent tails

cultured cells

fixed tissue

insects

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Genomic DNA Purification （*e.g.,* Tissue & Whole Blood）

1	Prepare sample	Cut 25mg into small pieces & 200ul whole blood
2	Pre-lyse sample	 **Tissue**: 180 µL Buffer GTL; 20 µL proteinase K; 56°C incubate 1-3h. **Whole Blood**: 20 µL proteinase K; If sample material is＜200µL; fill up volume to 200ul with PBS
3	Lyse sample	 200 µL Buffer GL; 70°C 10 Min
4	Adjust DNA Binding conditions	 200 µL 95 – 100% ethanol
5	Bind DNA	 LOAD ALL; 8,000 X *G*; 1 Min
6	Wash silica membrane	 1 wash 500ul GW1; 2 Wash 600ul GW2; 8,000 X *G*，1 Min
7	Dry silica membrane	10,000 X *G*; 2 Min
8	Elute highly pure DNA	 200 µL GE(70°C); RT; 1 Min; 10,000 X *G*; 1 Min

Kit Contents

Genorise General DNA Mini Kit	100
Cat No.	GERC-101076-100
DNA binding columns	100
Collection Tubes	100
Buffer GTL	22ml
Buffer G L	25ml
Buffer GW1	38ml +24ml ethanol
Buffer GW2	38ml +92ml ethanol
Buffer GE	25ml
Proteinase K(20mg/ml)	1.25ml*2

6. Troubleshooting

NO or poor DNA yield

①Incomplete lysis

- ●Sample not thoroughly homogenized and mixed with Buffer GL/Proteinase K .The mixture has to be vortexed vigorously immediately after the addition of Buffer GL.
- ●Cut tissue into small pieces before digestion and lysis.
- ●Increase incubation time with Proteinase K in either of two ways:
  (1). Incubate tissue with Proteinase K overnight.
  (2). Incubate with Proteinase K for 3 - 4 h, then add a fresh aliquot of Proteinase K (20 µL) and incubate another 1 - 2 h.
  ▲To accommodate increased volume (sample and enzyme), use 230 µL Buffer GL instead of 200 µL.
- ●Decreased Proteinase K activity:Store dissolved Proteinase K at -20℃.

②Ethanol not added to Buffer GW1 and Buffer GW2

- ● Add absolute ethanol to the buffers before using.
- ● After adding ethanol, mix the buffers well and store at +15 to +25℃.
- ●Always mark Buffer GW1 vial and Buffer GW2 vial to indicate whether ethanol has been added or not.

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 the Wash Buffer.

- ●.After the last wash step, make certain flowthrough solution containing Wash Buffer does not contact the bottom of the Filter Tube.
- ● If this has occurred, empty the Collection Tube and reinsert the contaminated filter, and re-centrifuge for 30 seconds.

Low yield from bacteria or yeast.

Cells are not lysed efficiently with lysozyme (bacteria) or lyticase (yeast).

- ●Make sure that your cells can be lysed by lysozyme or lyticase.
- ●Use alternative lysis procedures, such as mechanical grinding, vortexing with glass beads, boiling or repeated freeze-thaw.

Degraded DNA from tissue samples.

Nuclease activity in unlysed tissue.

- ●Tissue should be frozen (15 to 25℃) from the time of harvest until the lysis procedure starts.
- ●Use only small pieces of tissue (20 - 40 mg), in the procedure or homogenize tissue sample.
- ●Use alternative lysis procedures, such as mechanical grinding, vortexing with glass beads, boiling or repeated freeze-thaw.

Eluate from blood is still slightly colored.

Incomplete wash.

- ●Wash Filter Tube until flowthrough is colorless.
- ●Repeat purification by using 200 µL eluate from the first purification round as starting material; add 200 µL Binding Buffer, mix well, then add 200 µL ethanol（95-100%）.
- ●Follow corresponding procedure, starting with the application of the sample to the DNA binding column.
- ▲Omit Proteinase K digestion and 70℃ incubation.

4.6 Protocol for Washing and Elution

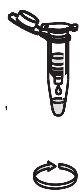
①After centrifugation:
- ●Remove the DNA binding column from the Collection Tube, discard the flowthrough liquid.
- ●Combine the DNA binding column with the Collection Tube.
- ●Add 500 μL **Buffer GW1**. Removal Buffer to the upper reservoir of the Filter Tube.
- ●Centrifuge 1 min at 8,000×g.

②●Remove the DNA binding column from the Collection Tube, discard the flowthrough liquid.
- ●Combine the DNA binding column with the Collection Tube.
- ●Add 600μL **Buffer GW2** to the upper reservoir of the DNA binding column.
- ●Centrifuge 1 min at 8,000×g and discard the flowthrough.

③●Remove the DNA binding column from the Collection Tube, discard the flowthrough liquid.
- ●Combine the DNA binding column with the Collection Tube.
- ●Add 600 μL **Buffer GW2** to the upper reservoir of the DNA binding column.
- ●Centrifuge 1 min at 8,000×g and discard the flowthrough.

④After discarding the flowthrough liquid:
- ●Centrifuge the DNA binding column for additional 2 min at full speed.
- ●Discard the Collection Tube.
- ▲The extra centrifugation time ensures removal of residual Wash Buffer.

⑤To elute the DNA:
- ●Insert the DNA binding column into a clean, sterile 1.5 ml microcentrifuge tube.
- ●Add 200 μL prewarmed **Buffer GE** to the upper reservoir of the DNA binding column.
- ●Centrifuge the tube assembly for 1 min at 10,000×g 1min.
- ▲Elution with 100μL (instead of 200μL) increases the final DNA concentration in the eluate , but also decreases the overall DNA yield.
- ▲Warm up the **Buffer GE** to 70℃ increases the final DNA concentration in the eluate.
- ▲If you wish to remove RNA from the eluted DNA treat your sample as follows:
  Add to the RNase A 2μL (10mg/ml) and incubate as appropriate(37℃,15min).



⑥The microcentrifuge tube now contains the eluted DNA.
Either use the eluted DNA directly or store the eluted DNA at +2 to +8℃ or 15 to 25℃ for later analysis.

5. Results

●**Purity:** Purified nucleic acids are free of other cellular components and DNA polymerase inhibitors.
▲RNA can be removed from purified DNA with an optional RNase digestion.

Expected Yield Variable depending on sample type. The table below shows experimental results:

Sample	Amount	Yield(ug) Total nucleic acids
Human whole blood	200 μL	4-12
Buffy coat	200 μL	20
Cultured cells	10^6 cells	15-20
Calf thymus	25mg	5-10
Mouse tail	0.2-0.5cm(25-50mg)	5-10
Bacterial cells	10^9	1-3
Yeast	10^8	10-13

Table of Contents

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1. Introduction

●**Genomic DNA Easy mini Kit** is designed for rapid,small-scale preparation of highly pure genomic DNA form any tissue, Animal Blood cells,bacteria,yeast.The purified DNA can be used directly for PCR,Southern blotting,or any kind of enzymatic reactions.

●The kit allows purification of up to 35 ug of pure genomic DNA with an A260/A280 ratio between 1.7 and 1.9,and is up to 50kb in size,with fragments of 30 kb predominating.The DNeasy procedure also efficiently recovers DNA fragments as small as 100 bp.

2.Storage

●DNA binding columns,and all buffers should be stored dry ,at room temperature(15-25°C) and are stable for 1 year under these conditions.

●Genomic DNA Easy mini kit contain a ready-to-use proteinase K solution, which is supplied in a specially formulated storage buffer. Proteinase K is stable for at least 1 year after delivery when stored at room temperature. For storage longer than one year or if ambient temperatures often exceed 25°C,we suggest storing proteinase K at 2-8°C

3. Equipment and Reagent to Be Supplied by User

Refer to the list below for additional reagents and equipment required for all isolation procedures: ●Absolute ethanol（95-100%）

For the isolation of mammalian whole blood, buffy coat, or cultured cells: ●PBS

For the isolation of mouse tail: ●1 ml disposable syringe without needle

For the isolation of nucleic acids from bacteria or yeast
- ●PBS
- ●Lysozyme [10 mg/ml in 10 mM Tris-HCl, pH 8.0]
- ●Lyticase (0.5 mg/ml)

For the isolation of nucleic acids from formalin-fixed paraffin-embedded tissue sections:
- ●Xylene
- ●Ethanol, 100%, 80%, 60%, 40%

RNase treatment (optional): ●RNase, DNase-free

Heating block or water bath

4. DNA Purification Protocols

4.1 Sample Material

● 200 μL mammalian whole blood ● 200 μL buffy coat ●10^4-10^6 cultured mammalian cells ●25 - 50 mg mammalian solid tissue
● 0.2 - 0.5 cm mouse tail (25 - 50 mg) ●10^8 yeast cells ●bacterial cells (gram positive or gram negative)
● Paraffin-embedded, fixed tissue sections

4.2 Purification of Total DNA from Animal Blood or Cells

①Prepare sample

If your sample volume is less than 200 µL, the sample volume should be expanded with PBS . Adjust the sample volume as follows:

Material	Action
cell culture cells	cell culture cells centrifuge medium with cells and resuspend cell pellet in 200µL PBS.
mammalian whole blood	If sample material is < 200 µL l fill up volume to 200 µL whole blood with PBS
Buffy coat	200 µL buffy coat

Tissue
180 µL Buffer GTL
20 µL proteinase K
56°C incubate 1-3h

Whole Blood
20 µL proteinase K
If sample material is＜200µL
fill up volume to 200ul with PBS

- 200 µL mammalian blood,
- 200 µL buffy coat,
- 10^6 cultured mammalian cells

▲Before starting the purification reaction, warm up the Elution Buffer to 70°C.


①

②To a nuclease free 1.5 ml microcentrifuge tube
- ●Add 200 µL of sample material.
- ●Add 200 µL **Buffer GL**. ●Add 20 µL **Proteinase K** (20mg/ml).
- ●Mix immediately and incubate at 70°C for 10 min.


②

200 µL Buffer GL
70°C 10 Min

③Add 200 µL 95 – 100% ethanol.

④●Insert one DNA binding column in one Collection Tube.
- ●Pipet the sample into the upper buffer reservoir of the DNA binding column.
- ●Insert the DNA binding column assembly into a standard tabletop centrifuge.
- ●Centrifuge 1 min at 8,000×g.


③

200 µL 95 – 100% ethanol

⑤ Proceed to Washing and Elution **section 4.6.**

4.3 Purification of Total DNA from Animal tissue

①●To a nuclease-free 1.5 ml microcentrifuge tube.
- ●Add 25 - 50 mg of sample material.
- ●Add 200 µl Buffer GTL. ●20 µl **Proteinase K**.
- ●Mix immediately and incubate for 1-3 h at 56 °C or until tissue is digested completely.
- ●For **rodent tail**，6-8h. if it is more convenient,samples can be lysed overnight.

▲The yield of nucleic acids can be increased by cutting the sample with a scalpel in small pieces before incubation.


④

Load all
8,000 X G
1 Min

②●Add 200 µl **Buffer GL**.
- ●Mix immediately and incubate at 70°C for 10 min.

③Add 200 µL 95 – 100% ethanol.

④●Insert one DNA binding column in one Collection Tube.
- ●Pipet the sample into the upper buffer reservoir of the Filter Tube.
- ●Insert DNA binding column assembly into a standard tabletop centrifuge.
- ●Centrifuge 1 min at 8,000×g.

⑤ Proceed to Washing and Elution **section 4.6.**

4.4 Purification of Total DNA from Fixed Paraffin-embedded Tissue

①Soak the tissue section in xylene to deparaffinized for approx. 30 min. Incubation time depends on the thickness of the section.

②Incubate the tissue section in a graded ethanol series for 10 s each:
The section should turn white after it is transferred to ethanol.
- ●100% ethanol (dehydration)/ 80% ethanol/ 60% ethanol/ 40% ethanol. ●Double distilled water (rehydration) for 10 s.

③●While viewing the section under a microscope, cut the desired tissue area from the rehydrated section with a scalpel.
- ●Transfer the sample to a clean, sterile, pre weighted 1.5 ml microcentrifuge tube.
- ●Determine the weight of the sample.

④To the tissue sample (25 - 50 mg)
- ●Add 200 µL **Buffer GTL**. ●Add 20 µL **Proteinase K** (reconstituted). ●Mix and incubate at overnight 37℃.

⑤●Add 20 µL **Proteinase K** (reconstituted). ●Incubate for 1 - 2 h at 56℃.
After this incubation step, no crude tissue particles should be visible.

⑥●Add 200 µL **Buffer GL** and mix thoroughly. ●Mix thoroughly and incubate 10 min at 70℃.

⑦●Add 200 µL ethanol（95-100%）and mix well.
- ●Use an automatic pipette to draw part of the sample into a 1 ml pipette tip.

This treatment draws insoluble tissue segments into the pipette tip and blocks it.
- ●Withdraw the pipette tip, carrying the insoluble tissue segments with it.

⑧●Insert one DNA binding column in one Collection Tube.
- ●Pipet the remainder of the liquid sample into the upper buffer reservoir of the Filter Tube.
- ●Insert the DNA binding column assembly into a standard tabletop centrifuge and centrifuge 1 min at 8,000×g.

⑨Proceed to Washing and Elution **section4.6.**

4.5 Purification of Total DNA from bacteria or yeast

①To a nuclease-free 1.5 ml microcentrifuge tube
- ●Add 200 µL-500 µL bacteria or yeast cells.
- ●Centrifuge for 5 min at 3,000×g.
- ●Resuspend cell pellet in 200µL PBS.

②**For Bacteria**: Add and incubate 5 µL lysozyme (10 mg/ml in 10 mM Tris-HCl, pH 8.0) and incubate 15 min at 37℃.
For Yeast: Add and incubate 10 µL lyticase (0.5 mg/ml) and incubate 30 min at 37℃.

③To the sample material
- ●Add 200 µL **Buffer GL**. ●Add 20 µL **Proteinase K** (reconstituted). ●Mix immediately and incubate for 10 min at 70℃.

④Add 200 µL ethanol（95-100%）and mix well.

⑤●Insert one DNA binding column in one Collection Tube.
- ●Pipet the liquid sample into the upper buffer reservoir of the Filter Tube.
- ●Insert the DNA binding column assembly into a standard tabletop centrifuge.
- ●Centrifuge 1 min at 8,000×g.

⑥Proceed to Washing and Elution section 4.6。