thermo scientific — USER GUIDE

Genomic DNA Purification Kit

Catalog Number K0512

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Genomic DNA Purification Kit is a simple and rapid system for high quality genomic DNA purification from various sources including: whole blood, serum, cell lines, bacterial cells, plant and mammalian tissues. The kit is based on selective detergent-mediated DNA precipitation from crude lysate. The entire procedure is rapid taking only 20–25 minutes with a typical yield of 2–10 µg genomic DNA from 0.2 mL of blood.

High molecular weight genomic DNA, 50 kb average from fresh blood, purified with the kit is suitable for direct use in all common molecular biology applications: PCR, Thermo Scientific™ FastDigest™ and conventional restriction digestion, cloning, DNA sequencing, and Southern blot analysis.

Contents and storage

Reagents that are provided in the kit are sufficient for 100 preparations.

Table 1 Genomic DNA Purification Kit (Cat. No. K0512)

Item	Amount	Storage
Lysis Solution	40 mL	15–25°C (room temperature)
Precipitation Solution (10× concentrated solution)	8 mL	
NaCl Solution (1.2 M sodium chloride)	10 mL	

Warnings and precautions

When handling blood samples, follow recommended procedures for biohazardous materials.

Guidelines for sample preparation

IMPORTANT! Freezing and thawing samples multiple times should be avoided. Each cycle dramatically diminishes the yield of intact DNA.

Blood preparation

- Collect whole blood in EDTA- or citrate-coated tubes to prevent clotting and DNA degradation.

IMPORTANT! DNA extracted from heparinized blood cannot be used for PCR.

- For a yield of 2–10 µg of DNA, we recommend using 200 µL of fresh blood for DNA isolation.
- If a larger quantity of DNA is needed, lyse 500 µL of blood with 1 mL of water. Centrifuge the leukocytes at 2,500 × *g* for 2 minutes, then resuspend the pellet in 200 µL of TE buffer.
- Blood can be stored at 2–4°C for up to 2 months. For long-term storage, aliquot 200 µL per tube, then store at -20°C.

Sera preparation

- We recommend using fresh sera samples.
- If frozen sera samples are used, ensure the samples are frozen in 200-µL aliquots and stored at -20°C.

Cell culture preparation

- Collect cells in culture by centrifugation, then resuspend in 200 µL of TE buffer.
- For efficient DNA separation from cell components, do not use more than 0.4–0.6 × 10^6 cells.
- Do not thaw samples before adding Lysis Solution.

Epithelium cell preparation

- Collect epithelium cells by centrifugation at 2,500 × *g* for 3 minutes. Remove the supernatant, then resuspend the cell pellet in 200 µL of TE buffer.
- Do not thaw samples before adding Lysis Solution.

Tissue preparation

- Pulverize 25–30 mg of mammalian tissue (either fresh or frozen at -70°C until use) or 50–100 mg of plant tissue in liquid nitrogen with mortar and pestle. Place the powder in a 1.5-mL microcentrifuge tube, then resuspend in 200 µL of TE buffer.
- Do not thaw samples before adding Lysis Solution.

Bacterial culture preparation

- Centrifuge the bacterial culture at 5,000 × *g* for 10 minutes.
- Place 10–20 mg of bacterial cells (either fresh or frozen at -20°C until use) in a 1.5-mL microcentrifuge tube, then resuspend in 200 µL of TE buffer.

- Do not thaw samples before adding Lysis Solution.

Purify genomic DNA

Prepare fresh precipitation solution by mixing 720 µL of sterile deionized water with 80 µL of supplied 10× concentrated precipitation solution.

1. Mix 200 µL of sample with 400 µL of Lysis Solution, then incubate at 65°C for 5 minutes.

   Note: If a frozen sample is used, add the Lysis Solution before thawing, then incubate the sample at 65°C for 10 minutes with occasional inversion of the tube.

2. Immediately add 600 µL of chloroform. Gently emulsify by inversion (3–5 times), then centrifuge the sample at 9,400 × *g* for 2 minutes.

3. Transfer the upper aqueous phase containing DNA to a new tube.

4. Add 800 µL of freshly prepared 1X Precipitation Solution, then mix gently by several inversions at room temperature for 1–2 minutes.

5. Centrifuge at 9,400 × *g* for 2 minutes.

6. Completely remove the supernatant, but do not dry the DNA pellet. Dissolve the DNA pellet in 100 µL of NaCl solution by gentle vortexing.

   IMPORTANT! Ensure that the pellet is completely dissolved.

7. Add 300 µL of cold 100% ethanol, then let the DNA precipitate for 10 minutes at -20°C. Centrifuge the sample at 9,400 × *g* for 3-4 minutes to pellet the DNA.

8. Remove the ethanol, then wash the pellet once with 70% cold ethanol.

9. Dissolve DNA in 100 µL of sterile deionized water by gentle vortexing.

   Note:
   - For samples with low amounts of DNA, such as serum, prolong the DNA precipitation to 1–20 hours at -20°C, then decrease the volume of water that is used to dissolve the DNA to 20 µL.
   - For PCR applications, it is not necessary to wash the DNA sample with 70% ethanol.
   - If RNA-free genomic DNA is necessary, add RNase A (Cat. No. EN0531) to a final concentration of 0.2 mg/mL after completing step 6. Mix by vortexing, then incubate for 10 minutes at 37°C.

10. Determine DNA concentration spectrophotometrically or visually after electrophoresis in agarose gel. Check for the absence of RNA, then estimate the average length of obtained DNA.

    Note:
    - Usually 2–10 µg of DNA from 200 µL of fresh blood is obtained with an average length of about 50 kb.
    - In blood samples, DNA yield depends on the quantity of leukocyte cells.
    - Viral DNA from sera samples is not visible in agarose gel.
    - Use 1–5 µL of DNA solution for 50 µL of PCR.

Troubleshooting

Observation	Possible cause	Recommended action
Low DNA yield	Blood: Sample was frozen and thawed several times. Sample was stored at 2–4°C longer than 2 months. Blood clots were present in the sample.	Use a fresh blood sample for DNA purification.
	Cell culture: Too many cells were used for purification, and DNA pellet turned insoluble. Sample was frozen and thawed several times.	Reduce cell quantity by a factor of two or more.
		Use a fresh cell culture sample.
	Any sample: DNA pellet was not completely dissolved during step 6.	Use a fresh sample.
Degraded DNA	Any sample: Inappropriate storage conditions of the sample.	Follow guidelines for sample storage. See “Guidelines for sample preparation”.
RNA contamination present	Tissue, bacteria, or cell culture: RNase A was not added during DNA purification procedure.	Add RNase A for a final concentration 0.2 mg/mL to the sample prior to the addition of ethanol then incubate for 10 minutes at 37°C. See step 9
	Any sample: Inaccurate handling during step 3 or not making 1X precipitation solution correctly.	Adjust prepared DNA sample volume to 200 µL with H_2O and repeat all purification steps.
	Any sample: The precipitation solution was not fresh.	Use freshly prepared precipitation solution.
Enzymatic reactions not running	Blood: Heparinized blood was used.	Use EDTA or citrate treated blood.

Observation	Possible cause	Recommended action
Enzymatic reactions not running *(continued)*	Any sample: Residual reagents (ethanol, salts, etc.) present in prepared DNA, because of inaccurate handling during step 7, step 8, or step 9.	Precipitate DNA with ethanol, wash with cold 70% ethanol, and dissolve in sterile deionized water.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0012656 B00

Revision	Date	Description
B00	15 April 2024	• The document was updated to the current template, with associated updates to the warranty, trademarks, and logos. • The Certificate of Analysis section was removed from the document.
A.0	15 October 2012	New document for the Genomic DNA Purification Kit.