GeneJET™ Genomic DNA Purification Kit

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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 Thermo Scientific™ GeneJET™ Genomic DNA Purification Kit is designed for rapid and efficient purification of high-quality genomic DNA from various mammalian cell culture and tissue samples, whole blood, bacteria, and yeast. The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation.

The standard procedure takes less than 20 minutes following cell lysis and yields purified DNA of more than 30 kb in size. Isolated DNA can be used directly in PCR, Southern blotting and enzymatic reactions.

Technology overview

Depending on the starting material, samples are digested with Proteinase K in either the supplied Digestion or Lysis Solution. RNA is removed by treating the samples with RNase A. The lysate is then mixed with ethanol and loaded on the purification column where the DNA binds to the silica membrane. Impurities are effectively removed by washing the column with the prepared wash buffers. Genomic DNA is then eluted under low ionic strength conditions with the Elution Buffer.

Table 1 Typical genomic DNA yields from various sources

Source	Amount	DNA yield
Mammalian blood	200 µL	4–6 µg
Mouse heart	10 mg	10–15 µg
Mouse tail	0.5 cm	8–10 µg
Rat liver	10 mg	10–20 µg
Rat spleen	5 mg	20–30 µg
Rat kidney	10 mg	25–30 µg
Rabbit ear	20 mg	5–10 µg
Bacillus pumilis cells	2×10^9 cells	10–15 µg
Escherichia coli cells	2×10^9 cells	10–15 µg
HeLa cells	2×10^6 cells	15–20 µg
Jurkat cells	5×10^6 cells	25–30 µg
Saccharomyces cerevisiae cells	1×10^8 cells	3–5 µg

Contents and storage

IMPORTANT! Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

Item	Cat. No. K0721 (50 reactions)	Cat. No. K0722 (250 reactions)	Storage
Proteinase K Solution	1.2 mL	5 × 1.2 mL	• Upon receipt, store the unopened vial at 15–25°C. • After use, store the vial at –20°C.
RNase A Solution	1.2 mL	5 × 1 mL	
Digestion Buffer	11 mL	55 mL	15–25°C
Lysis Solution	24 mL	2 × 60 mL	
Wash Buffer 1 (concentrated)	10 mL	40 mL	
Wash Buffer 2 (concentrated)	10 mL	40 mL	
Elution Buffer (10 mM Tris-Cl, pH 9.0, 0.1 mM EDTA)	30 mL	150 mL	
Collection Tubes (2 mL)	50	250	
GeneJET™ Genomic DNA Purification Columns and Collection Tubes	50	250	Store at 15–25°C for up to 6 months. For longer periods, store at 2–8°C.

Procedural guidelines

IMPORTANT! Wear gloves when handling the Lysis Solution and Wash Buffer I as these reagents contain irritants.

To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples and perform extractions from fresh material or material that has been immediately frozen and stored at -20°C or -70°C.

Before the first use of the kit

1. Add the indicated volume of ethanol (96-100%) to Wash Buffer I (concentrated) and Wash Buffer II (concentrated) prior to first use:

–	Cat. no. K0721 (50 reactions)		Cat. no. K0722 (250 reactions	
–	Wash Buffer I	Wash Buffer II	Wash Buffer I	Wash Buffer II
Concentrated wash solution	10 mL	10 mL	40 mL	40 mL
Ethanol (96–100%)	30 mL	30 mL	120 mL	120 mL
Total Volume	40 mL	40 mL	160 mL	160 mL

2. After the ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

Before each use

Check the Digestion Solution and Lysis Solution for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use.

Prepare buffers

Prepare buffers as described in the following table.

Buffer	Components
Mammalian cell lysate preparation	• PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4, pH 7.4) • TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
Gram-positive bacteria lysate preparation	• Gram-positive bacteria lysis buffer (20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100) • Add lysozyme to 20 mg/mL immediately before use
Yeast lysate preparation	Yeast lysis buffer (5 mg/mL zymolyase 20T, 1 M sorbitol, 0.1 M EDTA)

Purify genomic DNA from mammalian tissue or rodent tails

1. Grind up to 20 mg of mammalian tissue, 10 mg of spleen tissue, 0.6 cm of rat tail clip, or 0.5 cm mouse tail clip in liquid nitrogen using a mortar and pestle.

   Note: Alternatively, cut the tissue into small pieces or disrupt it using a homogenizer.

2. Collect the tissue into a 1.5 mL microcentrifuge tube then resuspend in 180 µL of Digestion Solution.

3. Add 20 µL of Proteinase K Solution then mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

4. Incubate the sample at 56°C until the tissue is completely lysed and no particles remain. During the incubation, vortex the vial occasionally or use a shaking water bath, rocking platform, or thermomixer.

   Table 2 Recommended incubation times

Quantity	Recommended incubation time
5 mg of tissue (except spleen)	1 hour
10 mg of tissue (except spleen)	2 hours
20 mg of tissue (except spleen)	3 hours
5 mg of spleen tissue	2 hours
10 mg of spleen tissue	3 hours
Mouse tail (0.5 cm), rat tail (0.6 cm)	6 hours

   Note: Lysis time varies on the type and amount of tissue processed. In some cases, the incubation time should be prolonged to 6–8 hours or overnight (for rodent tail) until complete lysis occurs.

5. Add 20 µL of RNase A Solution, then mix by vortexing. Incubate at room temperature for 10 minutes.

6. Add 200 µL of Lysis Solution. Mix thoroughly by vortexing for 15 seconds until a homogeneous mixture is obtained.

7. Add 400 µL of 50% ethanol, then mix by pipetting or vortexing.

8. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution.

9. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube.

   Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

10. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through and place the purification column back into the collection tube.

11. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

12. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again for 1 minute at ≥12,000 × *g*.

13. Discard the collection tube containing the flow-through solution then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

14. To elute genomic DNA, add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane. Incubate at room temperature for 2 minutes then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, <5 mg of tissue) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

15. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from cultured mammalian cells

1. Collect suspension cells or adherent cells using the following methods:

    - **Suspension cells**: Collect up to 5 × 10^6 cells in a centrifuge tube. Pellet cells by centrifugation at 250 × *g* for 5 minutes. Discard the supernatant. Rinse cells once with PBS to remove remaining medium, then repeat the centrifugation step. Discard the supernatant.
    - **Adherent cells**: Remove the growth medium from a culture plate containing up to 2 × 10^6 cells. Rinse cells once with PBS to remove residual medium. Discard PBS. Detach the cells from the culture plate by scraping in an appropriate volume of PBS or by trypsinization. Transfer the cells to a microcentrifuge tube then pellet them by centrifugation at 250 × *g* for 5 minutes. Discard supernatant.

2. Resuspend the collected cells in 200 µL of TE buffer or PBS.

3. Add 200 µL of Lysis Solution and 20 µL of Proteinase K Solution to the cell pellet. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

4. Incubate the sample at 56°C for 10 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.

5. Add 20 µL of RNase A Solution, then mix by vortexing. Incubate the mixture at room temperature for 10 minutes.

6. Add 400 µL of 50% ethanol, then mix by pipetting or vortexing.

7. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.

8. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

    Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

9. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

10. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

11. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12,000 × *g* for 1 minute.

12. Discard the collection tube containing the flow-through solution then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

13. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes, then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

14. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from mammalian blood

1. Add 400 µL of Lysis Solution and 20 µL of Proteinase K Solution to 200 µL of whole blood. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

2. Incubate the sample at 56°C for 10 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.

3. Add 200 µL of ethanol (96–100%), then mix by pipetting or vortexing.

4. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.

5. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

   Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

6. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

7. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

8. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12,000 × *g* for 1 minute.

9. Discard the collection tube containing the flow-through solution then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

10. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes, then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

11. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from gram-negative bacteria

1. Harvest up to 2 × 10^9 bacterial cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation at 5,000 × *g* for 10 minutes. Discard the supernatant.

2. Resuspend the pellet in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution, then mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

3. Incubate the sample at 56°C for 30 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.

4. Add 20 µL of RNase A Solution. Mix by vortexing, then incubate the mixture at room temperature for 10 minutes.

5. Add 200 µL of Lysis Solution to the sample. Mix thoroughly by vortexing for about 15 seconds until a homogeneous mixture is obtained.

6. Add 400 µL of 50% ethanol then mix by pipetting or vortexing.

7. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.

8. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

   Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

9. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

10. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

11. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12,000 × *g* for 1 minute.

12. Discard the collection tube containing the flow-through solution then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

13. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

14. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from gram-positive bacteria

Prepare Gram-positive bacteria lysis buffer with 20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100. Add lysozyme to 20 mg/mL immediately before use.

1. Harvest up to 2 × 10^9 bacterial cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation at 5,000 × *g* for 10 minutes. Discard the supernatant.

2. Resuspend the pellet in 180 µL of gram-positive bacteria lysis buffer. Incubate at 37°C for 30 minutes.

3. Add 200 µL of Lysis Solution and 20 µL of Proteinase K. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

4. Incubate the sample at 56°C for 30 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.

5. Add 20 µL of RNase A Solution. Mix by vortexing then incubate the mixture at room temperature for 10 minutes.

6. Add 200 µL of Lysis Solution to the sample. Mix thoroughly by vortexing for about 15 seconds until a homogeneous mixture is obtained.

7. Add 400 µL of 50% ethanol then mix by pipetting or vortexing.

8. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.

9. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

   Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

10. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

11. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

12. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12,000 × *g* for 1 minute.

13. Discard the collection tube containing the flow-through solution, then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

14. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

15. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from yeast

1. Harvest up to 1 × 10^8 yeast cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation at ≥12,000 × *g* for 5–10 seconds. Discard the supernatant.

2. Resuspend the pellet in 500 µL of Yeast lysis buffer. Incubate at 37°C for 1 hour.

3. Centrifuge cells at 3,000 × *g* for 10 minutes. Discard the supernatant.

4. Resuspend the pellet in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution then mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

5. Incubate the sample at 56°C for 45 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.

6. Add 20 µL of RNase A Solution. Mix by vortexing then incubate the mixture at room temperature for 10 minutes.

7. Add 200 µL of Lysis Solution to the sample. Mix thoroughly by vortexing for about 15 seconds until a homogeneous mixture is obtained.

8. Add 400 µL of 50% ethanol then mix by pipetting or vortexing.

9. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.

10. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

    Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

11. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

12. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12,000 × *g* for 3 minutes.

13. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12,000 × *g* for 1 minute.

14. Discard the collection tube containing the flow-through solution, then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.

15. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with an additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (for example, ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be decreased to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.

16. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Purify genomic DNA from buccal swabs

1. To collect a sample, scrape the swab 5–6 times against the inside cheek.
2. Swirl the swab for 30–60 seconds in 200 µL of 1 × PBS.
3. Add 400 µL of Lysis Solution and 20 µL of Proteinase K Solution to 200 µL of whole blood. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
4. Incubate the sample at 56°C for 10 minutes. Vortex occasionally or use a shaking water bath, rocking platform, or thermomixer until the cells are completely lysed.
5. Add 200 µL of ethanol (96–100%), then mix by pipetting or vortexing.
6. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube.
7. Centrifuge the column at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution. Place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube .

   Note: Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.
8. Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
9. Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET™ Genomic DNA Purification Column. Centrifuge at ≥12000 × *g* for 3 minutes.
10. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, then centrifuge the column again at ≥12000 × *g* for 1 minute.
11. Discard the collection tube containing the flow-through solution, then transfer the GeneJET™ Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube.
12. Add 200 µL of Elution Buffer to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA. Incubate at room temperature for 2 minutes, then centrifuge at 8,000 × *g* for 1 minute.

    Note:
    - For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer.
    - If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g. ≤1 × 10^6 of cultured mammalian cells) the volume of the Elution Buffer added to the column can be reduced to 50–100 µL. Smaller volumes of Elution Buffer, however, can result in a smaller final quantity of eluted DNA.
13. Discard the purification column.

    Note: Use the purified DNA immediately in downstream applications or store at -20°C.

Troubleshooting

Observation	Possible cause	Recommended action
Low yield of purified DNA	Excess sample was used during lysate preparation.	Reduce the amount of starting material. Do not use more blood than indicated in lysis protocols.
	The starting material was not completely digested.	Extend the Proteinase K digestion at 56°C until complete lysis occurs and no particles remain visible in solution.
	Ethanol was not added to the lysate.	Ensure that ethanol is added to the lysate before applying the sample to the Purification Column.
	Ethanol was not mixed with the lysate.	After the addition of ethanol to the lysate, mix the sample by vortexing or pipetting.
	Ethanol was not added to Wash Buffers.	Ensure that ethanol is added to Wash Buffer WB I and Wash Buffer II before use. See “Before the first use of the kit” on page 2.

Observation	Possible cause	Recommended action
Low yield of purified DNA *(continued)*	Columns stored at room temperature (15–25°C) for longer than 6 months.	Extended room temperature storage (15–25°C) for columns beyond 6 months may lead to membrane drying and reduced DNA yield. For prolonged storage exceeding 6 months, it is recommended to store columns at 2–8°C. See "Contents and storage" on page 2.
Purified DNA is degraded	Sample was frozen and thawed repeatedly.	Avoid repeated sample freeze/thaw cycles. Use a fresh sample for DNA isolation. Perform extractions from fresh material when possible.
	Inappropriate sample storage conditions.	Store mammalian tissues at -70°C and bacteria at -20°C until use. Whole blood can be stored at 4°C for no longer than 1–2 days. For long term storage blood samples should be aliquoted in 200 µL portions and stored at -20°C.
RNA contamination	RNase A treatment was not carried out.	Carry out RNase A treatment step described in the purification procedure.
Column becomes clogged during purification	Excess sample was used during lysate preparation.	Reduce the amount of starting material. A maximum of 2×10^9 of bacteria cells, 5×10^6 of suspension cells and 20 mg of mammalian tissue is recommended for lysate preparation.
	Tissue was not completely digested.	Extend the Proteinase K digestion at 56°C until complete lysis occurs and no particles remain.
Inhibition of downstream enzymatic reactions	Purified DNA contains residual ethanol.	If residual solution is seen in the purification column after washing the column with Wash Buffer II, empty the collection tube then re-spin the column at ≥12,000 × *g* for 1 minute.
	Purified DNA contains residual salt.	Use the correct order for the Washing Buffers. Always wash the purification column with Wash Buffer I first and then proceed to washing with Wash Buffer II.

Documentation and support

Customer and technical support

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- 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

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Revision history: Pub. No. MAN0012633 C00

Revision	Date	Description
C00	17 April 2024	• The document was updated to the current template, with associated updates to the warranty, trademarks, and logos. • The storage conditions for columns and collection tubes were updated.
B.0	7 November 2016	Updated information in manual.
A.0	17 October 2015	New document for the GeneJET™ Genomic DNA Purification Kit.

The information in this guide is subject to change without notice.