JetFlex Genomic DNA Purification Kits

For fast and reliable purification of genomic DNA from blood, mammalian cells, tissue, buffy coat, and all types of body fluids

Cat. nos. 600100 and 600500

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User Manual

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

Table of Contents

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Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Quantity	Cat. no.
JetFlex Genomic DNA Purification Kit	100	600 100
	500	600 500

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Shipping and Storage

Each kit is shipped at room temperature.

Upon receipt, store all buffers at room temperature and store all enzymes at 4°C.

Note: For long-term storage, aliquot RNase A in single use tubes and store at –20°C.

All components are guaranteed stable for 6 months when stored properly.

Continued on next page

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Kit Contents and Storage, Continued

Kit Contents

The components included in the JetFlex genomic DNA (gDNA) Purification Kits are listed below.

Reagents	100 preps	500 preps
	600 100	600 500
Red Blood Cell Lysis Buffer (RBC)	33 mL	165 mL
Cell Lysis Buffer (CLB)	33 mL	165 mL
Protein Precipitation Buffer (PPT)	17 mL	83 mL
Pellet Compactor	8 mL	30 mL
DNA Dissolving Buffer (TE)	22 mL	110 mL
Proteinase K (lyophilized powder)	2 × 21 mg	10 × 21 mg
RNase A (4 mg/mL)	1 mL	5 × 1 mL

Note

Sufficient reagents are included in the kit for 100 or 500 preparations. For larger starting sample volumes, the number of preparations will be decreased and for smaller starting sample volumes, the number of preparations per kit will be increased.

Continued on next page

Kit Contents and Storage, Continued

Buffer Composition

The composition of buffers included in the JetFlex gDNA Kits is listed below.

Buffer	Composition
Red Blood Cell Lysis Buffer	Proprietary Formulation
Cell Lysis Buffer	Proprietary Formulation
Protein Precipitation Buffer	Proprietary Formulation
Pellet Compactor	Proprietary Formulation
DNA Dissolving Buffer (TE)	10 mM Tris-HCl, 0.1 mM EDTA, pH 8.5
Proteinase K	Lyophilized powder
RNase A	4 mg/mL in 50 mM Tris-HCl, 10 mM EDTA, pH 8.0

Introduction

System Overview

Introduction

The JetFlex Genomic DNA Purification Kits allow for the rapid and efficient purification of high quality, high molecular weight, RNA-free genomic DNA (gDNA). The kits are designed to efficiently isolate genomic DNA from mammalian cells and tissues, blood samples, plant tissue, bacteria, and yeast in a single tube without the use of spin columns or a vacuum manifold. The procedure is readily scaled up or down, making it adaptable for a wide variety of starting sample volumes. DNA up to 150 kb in size may be purified.

System Summary

Genomic DNA is purified with the JetFlex gDNA Purification Kits by lysing nuclei with a cell lysis buffer and if necessary, treating the sample with RNase A and Proteinase K to degrade RNA and proteins. DNA is then precipitated under high salt conditions to remove impurities. Following ethanol removal, the DNA is redissolved in the DNA Dissolving Buffer (TE).

Advantages

The advantages of using JetFlex Genomic DNA Purification Kits are:

- Efficient purification of genomic DNA up to 150 kb from a variety of samples such as mammalian cells and tissues, blood samples, body fluids, plant tissue, bacteria, and yeast
- Rapid purification of high-quality DNA within 30 minutes without using any organic or toxic reagents
- Minimal contamination from RNA
- Reliable performance of the purified DNA in PCR, restriction enzyme digestion, and Southern blotting

Continued on next page

System Overview, Continued

Sample treatment and DNA Yield

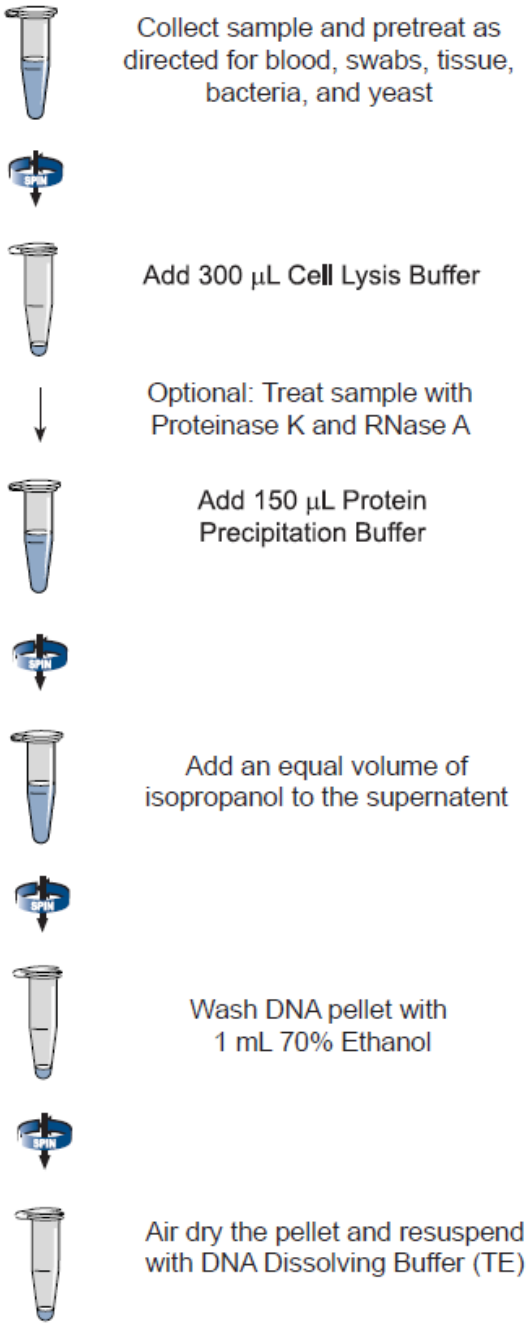
The table below lists the expected yield and necessary sample treatment according to the starting material.

Species & Material	Typical DNA yield	Proteinase K treatment	RNase A treatment	Page no.
Human Whole Blood • 300 µL • 2 mL • 10 mL • 50 mL	 • 5–10 µg • 50–100 µg • 250–500 µg • 1.25–2.5 mg	Optional	Optional	6
Non-Mammalian Blood	2.5–7.5 mg/mL	Optional	Required	6
Cultured Cells	10–30 µg per 3×10^6 cells	Optional	Required	11
Animal Tissue	20–150 µg per 10 mg	Required	Required	15
Plant Tissue	10–15 µg per 40 mg	Required	Required	15
Body Fluids	2–50 µg/mL	Optional	Optional	18
Swabs (Buccal, Pharyngeal, Nasal, Vaginal, etc.)	0.5–3.5 µg	Optional	Optional	23
Gram-Negative Bacteria	20 µg/mL of culture	Optional	Required	27
Gram-Positive Bacteria	10 µg/mL of culture	Optional	Required	27
Yeast	5–7 µg/mL	No	Required	31

Experimental Outline

Introduction

The figure below illustrates the basic steps necessary to use the kit.



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Methods

General Information

Follow the recommendations below to obtain the best results:

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases and ensure that no DNases are introduced into the sterile solutions of the kit
- Make sure all equipment that comes in contact with DNA is sterile, including pipette tips and microcentrifuge tubes
- To minimize DNA degradation, perform lysate preparation steps quickly, and avoid repeated freezing and thawing of DNA samples
- Perform all centrifugation steps at room temperature
- Incubate sample overnight with DNA Dissolving Buffer

Safety Information

Follow the safety guidelines below when using the kit.

- Treat all reagents supplied in the kit as potential irritants.
- Wear a suitable lab coat, disposable gloves, and goggles.
- Handle all blood and tissue samples in compliance with established institutional guidelines and take the appropriate precautions (wear a laboratory coat, disposable gloves, and eye protection). Since safety requirements for use and handling of blood and tissue samples may vary at individual institutions, consult the health and safety guidelines and/or officers at your institution.
- When processing blood and tissue samples, dispose of the waste appropriately as biohazardous waste.

Continued on next page

General Information, Continued

Before Starting

Resuspend Proteinase K in double-distilled or Milli-Q®-grade water to a final concentration of 20 mg/mL. Store the reconstituted enzyme in single-use aliquots at –20°C. Avoid repeated freezing and thawing.

Milli-Q® is a registered trademark of Millipore Corporation

Purifying gDNA from Blood

Starting Sample

JetFlex Genomic DNA Purification Kits are designed to purify genomic DNA from fresh or frozen whole blood, or buffy coat samples which may be collected in the presence of anti-coagulants such as EDTA, heparin, or citrate. Since EDTA also reduces DNA degradation, EDTA is the optimal anti-coagulant.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Materials Needed

- Sample for DNA isolation
- Sterile, DNase-free microcentrifuge tubes (for small preparations) or sterile, DNase-free 15 mL or 50 mL tubes (for larger sample preparation)
- Vortex mixer (multi-tube vortexer recommended)
- Water baths or heat blocks
- Centrifuge capable of centrifuging >12,000 × *g*
- Isopropanol
- 70% ethanol

Components supplied with the kit

- Red Blood Cell Lysis Buffer (RBC)
- Cell Lysis Buffer (CLB)
- Proteinase K (lyophilized powder, see **Before Starting**, page 5)
- RNase A (4 mg/mL)
- Protein Precipitation Buffer (PPT)
- DNA Dissolving Buffer (TE)

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Purifying gDNA from Blood, Continued

Preparing Blood Lysate

Prepare lysate from blood samples as described below.

1. Add fresh or frozen blood or buffy coat to an equal volume of RBC Lysis Buffer. Common starting volumes are shown in the table below.

Starting Sample Vol.	RBC Lysis Buffer Vol.
300 µL	300 µL
2 mL	2 mL
10 mL	10 mL
50 mL	50 mL

2. Mix well by inverting the tube until the mixture is homogenous.

   Note: If the blood sample is fresh (drawn within 1 hour), incubate at room temperature for one minute to ensure complete lysis of erythrocytes.

3. Centrifuge the sample according to the conditions in the table.

Centrifugation Conditions	Starting Sample Vol. 300 µL	Starting Sample Vol. ≥ 2 mL
Acceleration	12,000 × *g*	2,000 × *g*
Time	30 seconds	10 minutes

4. Remove the red supernatant, leaving behind a pellet of DNA-containing white blood cells. Bump vortex the white cell pellet vigorously to loosen the cells from the wall, which enhances cell lysis (next step).

Continued on next page

Purifying gDNA from Blood, Continued

Preparing Blood Lysate, Continued

5. Add an equal volume of Cell Lysis Buffer (CLB) to the disrupted cell pellet (i.e., for a 300 µL starting sample, add 300 µL CLB). Lyse the cells by pipetting up and down.

   Note: Usually no incubation is required. However, if cell clumps are visible after mixing, incubate at 37°C or room temperature until the lysate is homogeneous and clear. Samples are stable in CLB for several months at room temperature.

6. *Optional:* For every 300 µL starting sample volume, add 20 µL Proteinase K. Incubate at 58°C for 1 hour to overnight until the mixture is clear, indicating complete lysis.

   Note: The optional Proteinase K digestion is necessary if the blood sample is partially or completely clotted.

7. *Optional:* For every 300 µL starting sample volume, add 1.5 µL RNase A (i.e., add 1 µL RNase A to a 200 µL starting sample). Incubate at 37°C for 5 minutes.

8. Allow the lysate to cool to room temperature and then proceed directly to **Purifying gDNA**, next page.

Continued on next page

Purifying gDNA from Blood, Continued

Purifying gDNA

The following procedure is for purifying DNA from 300 µL blood lysate. To purify DNA from different starting sample volumes, scale up or down the buffer and reagent volumes proportionately.

1. Add 150 µL Protein Precipitation Buffer (PPT) to 300 µL of blood lysate from Step 8, previous page.
2. Mix thoroughly by vortexing for 20 seconds until a homogenous suspension is obtained.
3. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or insignificant, proceed to **Procedure for Pellet Compacting**, page 38.
4. Transfer the supernatant into a clean, sterile microcentrifuge tube.
5. Add an equal volume of isopropanol. Mix thoroughly by inverting the tube until the mixture is homogenous. Do not vortex.
6. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated DNA is visible as a white pellet.
7. Decant the supernatant. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.
8. Add 1 mL 70% ethanol and wash the DNA pellet by inverting the tube several times.
9. Centrifuge at 12,000 × *g* for 1 minute at room temperature.
10. Carefully decant the supernatant.

    Note: Be careful not to discard the DNA as the DNA pellet may be loosely adherent to the tube.
11. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.

Purifying gDNA from Mammalian Cells

Introduction

The JetFlex Genomic DNA Purification Kits are designed to purify genomic DNA from 1–2 × 10^6 to 1–2 × 10^7 mammalian cells.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Materials Needed

- Sample for DNA isolation
- Phosphate Buffered Saline (PBS), see page 40 for ordering information
- Sterile, DNase-free microcentrifuge tubes (for small preparations) or sterile, DNase-free 15 mL or 50 mL tubes (for larger sample preparation)
- Vortex mixer (multi-tube vortexer recommended)
- Water baths or heat blocks
- Centrifuge capable of centrifuging >12,000 × *g*
- Isopropanol
- 70% ethanol

Components supplied with the kit

- Cell Lysis Buffer (CLB)
- Proteinase K (lyophilized powder, see **Before Starting**, page 5)
- RNase A (4 mg/mL)
- Protein Precipitation Buffer (PPT)
- DNA Dissolving Buffer (TE)

Continued on next page

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Purifying gDNA from Mammalian Cells, Continued

Preparing Cell Lysate

Place cells on ice immediately before processing.

1. Collect 1–2 × 10^6 to 1–2 × 10^7 cells in a balanced salt solution (i.e., PBS).
2. Centrifuge the cells according the conditions in the table.

Centrifugation Conditions	Starting Sample 1–2 × 10^6 cells	Starting Sample 1–2 × 10^7 cells
Acceleration	12,000 × *g*	2,000 × *g*
Time	15 seconds	2 minutes

3. Remove the supernatant.
4. Add Cell Lysis Buffer (CLB) to the disrupted cell pellet.

Starting Cell Count	Buffer CLB
1–2 × 10^6	300 µL
1–2 × 10^7	3 mL

5. Lyse the cells by pipetting up and down.

   Note: Usually no incubation is required. However, if cell clumps are visible after mixing, incubate at 58°C until the lysate is homogeneous and clear. Samples are stable in CLB for several months at room temperature.

6. *Optional:* Add Proteinase K (20 mg/mL). Incubate at 58°C for 1 hour to overnight until the mixture is clear, indicating complete lysis.

Starting Cell Count	Proteinase K
1–2 × 10^6	20 µL
1–2 × 10^7	200 µL

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Purifying gDNA from Mammalian Cells, Continued

Preparing Cell Lysate, Continued

7. Add RNase A. Incubate at 37°C for 5 minutes.

Starting Cell Count	RNase A
1–2 × 10^6	10 µL
1–2 × 10^7	100 µL

8. Allow the lysate to cool to room temperature and then proceed directly to **Purifying gDNA**, below.

Purifying gDNA

The following procedure is for purifying DNA from 300 µL cellular lysate. To purify DNA from a different starting sample volume, scale up or down the buffer and reagent volumes proportionately.

1. Add 150 µL Protein Precipitation Buffer (PPT) to 300 µL of cellular lysate from Step 8, above.
2. Mix thoroughly by vortexing for 20 seconds until a homogenous suspension is obtained.
3. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or insignificant, proceed to **Procedure for Pellet Compacting**, page 38.
4. Transfer the supernatant into a clean, sterile microcentrifuge tube.
5. Add an equal volume of isopropanol. Mix thoroughly by inverting the tube until the mixture is homogenous. Do not vortex.
6. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated DNA is visible as a white pellet.

Purifying gDNA from Mammalian Cells, Continued

Purifying gDNA, Continued

7. Decant the supernatant. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.
8. Add 1 mL 70% ethanol and wash the DNA pellet by inverting the tube several times.
9. Centrifuge at 12,000 × *g* for 1 minute at room temperature.
10. Carefully decant the supernatant.

    Note: Be careful not to discard the DNA as the DNA pellet may be loosely adherent to the tube.
11. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.
12. Evaporate residual ethanol by incubating the sample at 50 °C to 55°C for 10 minutes.

    Note: If desired, you may evaporate residual ethanol at room temperature, but in this case, increase the incubation time.
13. After ethanol evaporation is complete (i.e., DNA pellet appears dry and the ethanol odor is undetectable), resuspend the DNA pellet in a suitable volume of DNA Dissolving Buffer (TE).
14. Incubate the tube at room temperature overnight or at 65°C for up to one hour to completely resuspend the DNA.

    The DNA should be completely dissolved as a clear, colorless solution. If precipitates are present, the A_{260}/A_{280} ratio is <1.70, or the DNA is contaminated with buffer, protein, or RNA, re-purify the DNA sample (see **Purifying Impure DNA,** page 39).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

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Purifying gDNA from Tissues, Continued

Preparing Tissue Lysate

The following procedure is for purifying DNA from up to 10 mg fresh, frozen, fixed, or paraffin-embedded tissue sample. To purify DNA from a larger tissue sample, scale up the buffer and reagent volumes proportionately.

1. Grind the tissue sample in liquid nitrogen with mortar and pestle. Keep the sample on ice to minimize DNase activity.
2. Add 300 µL Cell Lysis Buffer (CLB) to the tissue sample.

   Note: *Optional:* Homogenize the sample thoroughly using 30–50 strokes with a tube pestle.
3. Add 20 µL Proteinase K. Incubate at 58°C for 1 hour to overnight until the mixture is clear, indicating complete lysis.
4. Add 10 µL RNase A. Incubate at 37°C for 5 minutes.
5. Allow the lysate to cool to room temperature and then proceed directly to **Purifying gDNA,** below.

Purifying gDNA

1. Add 150 µL Protein Precipitation Buffer (PPT) to 300 µL of tissue lysate from Step 5, above.
2. Mix thoroughly by vortexing for 20 seconds until a homogenous suspension is obtained.
3. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or insignificant, proceed to **Procedure for Pellet Compacting**, page 38.
4. Transfer the supernatant into a clean, sterile microcentrifuge tube.
5. Add an equal volume of isopropanol. Mix thoroughly by inverting the tube until the mixture is homogenous. Do not vortex.

Continued on next page

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Purifying gDNA from Tissues, Continued

Purifying gDNA, Continued

6. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated DNA is visible as a white pellet.
7. Decant the supernatant. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.
8. Add 1 mL 70% ethanol and wash the DNA pellet by inverting the tube several times.
9. Centrifuge at 12,000 × *g* for 1 minute at room temperature.
10. Carefully decant the supernatant.

    Note: Be careful not to discard the DNA as the DNA pellet may be loosely adherent to the tube.
11. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.
12. Evaporate residual ethanol by incubating the sample at 50 °C to 55°C for 10 minutes.

    Note: If desired, you may evaporate residual ethanol at room temperature, but in this case, increase the incubation time.
13. After ethanol evaporation is complete (i.e., DNA pellet appears dry and the ethanol odor is undetectable), resuspend the DNA pellet in a suitable volume of DNA Dissolving Buffer (TE).
14. Incubate the tube at room temperature overnight or at 65°C for up to one hour to completely resuspend the DNA.

    The DNA should be completely dissolved as a clear, colorless solution. If precipitates are present, the A_{260}/A_{280} ratio is <1.70, or the DNA is contaminated with buffer, protein, or RNA, re-purify the DNA sample (see **Purifying Impure DNA,** page 39).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Purifying gDNA from Body Fluids

Introduction

JetFlex Genomic DNA Purification Kits are designed to purify genomic DNA from body fluids such as plasma, saliva, serum, milk, urine, cerebrospinal fluid, and sputum.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Materials Needed

- Sample for DNA isolation
- Sterile, DNase-free microcentrifuge tubes (for small preparations) or sterile, DNase-free 15 mL or 50 mL tubes (for larger sample preparation)
- Vortex mixer (multi-tube vortexer recommended)
- Water baths or heat blocks
- Centrifuge capable of centrifuging >12,000 × *g*
- Isopropanol
- 70% ethanol

Components supplied with the kit

- Cell Lysis Buffer (CLB)
- Proteinase K (lyophilized powder, see page 5)
- RNase A (4 mg/mL)
- Protein Precipitation Buffer (PPT)
- DNA Dissolving Buffer (TE)

Continued on next page

Purifying gDNA from Body Fluids, Continued

Preparing Body Fluid Lysate

1. Add 5 volumes of Cell Lysis Buffer (CLB) to 1 volume of body fluid (i.e., add 250 µL CLB to 50 µL body fluid). Lyse the cells immediately by pipetting up and down.

   Note: Samples may be fresh or frozen and may contain cellular debris. If the sample has a high protein content, increase the CLB ratio to 11:1 (i.e., 550 µL CLB for a 50 µL body fluid sample).

2. Incubate at 58°C until the lysate is homogeneous and clear.

3. *Optional:* Add 1 µL Proteinase K for every 2.5 µL of starting body fluid (i.e., add 20 µL Proteinase K to a 50 µL body fluid starting sample).

4. Incubate at 58°C for 1 hour to overnight until the mixture is clear, indicating complete lysis.

   Note: If any cellular debris or particulate matter is still present after lysis, centrifuge the sample and transfer the clear supernatant to a clean tube.

5. *Optional:* Add 1 µL RNase A for every 5 µL starting sample volume, (i.e., add 10 µL RNase A to a 50 µL body fluid starting sample). Incubate at 37°C for 5 minutes.

6. Allow the lysate to cool to room temperature and then proceed directly to **Purifying gDNA,** next page.

Continued on next page

Purifying gDNA from Body Fluids, Continued

Purifying gDNA

The following procedure is for purifying DNA from 300 µL cellular lysate. To purify DNA from a different starting sample volume, scale up or down the buffer and reagent volumes proportionately.

1. Add 150 µL Protein Precipitation Buffer (PPT) to 300 µL of cellular lysate from Step 6, previous page.
2. Mix thoroughly by vortexing for 20 seconds until a homogenous suspension is obtained.
3. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or insignificant, proceed to **Procedure for Pellet Compacting**, page 38.
4. Transfer the supernatant into a clean, sterile microcentrifuge tube.
5. Add an equal volume of isopropanol. Mix thoroughly by inverting the tube until the mixture is homogenous. Do not vortex.
6. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated DNA is visible as a white pellet.
7. Decant the supernatant. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.
8. Add 1 mL 70% ethanol and wash the DNA pellet by inverting the tube several times.
9. Centrifuge at 12,000 × *g* for 1 minute at room temperature.
10. Carefully decant the supernatant.

    Note: Be careful not to discard the DNA as the DNA pellet may be loosely adherent to the tube.

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Purifying gDNA from Body Fluids, Continued

Purifying gDNA, Continued

11. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.
12. Evaporate residual ethanol by incubating the sample at 50 °C to 55°C for 10 minutes.

    Note: If desired, you may evaporate residual ethanol at room temperature, but in this case, increase the incubation time.
13. After ethanol evaporation is complete (i.e., DNA pellet appears dry and the ethanol odor is undetectable), resuspend the DNA pellet in a suitable volume of DNA Dissolving Buffer (TE).
14. Incubate the tube at room temperature overnight or at 65°C for up to one hour to completely resuspend the DNA.

    The DNA should be completely dissolved as a clear, colorless solution. If precipitates are present, the A_{260}/A_{280} ratio is <1.70, or the DNA is contaminated with buffer, protein, or RNA, re-purify the DNA sample (see **Purifying Impure DNA,** page 39).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Purifying gDNA from Swabs

Introduction

The JetFlex Genomic DNA Purification Kits are designed to purify genomic DNA from swabs, including buccal, pharyngeal, nasal, and vaginal.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Materials Needed

- Sample for DNA isolation
- Sterile, DNase-free microcentrifuge tubes (for small preparations) or sterile, DNase-free 15 mL or 50 mL tubes (for larger sample preparation)
- Vortex mixer (multi-tube vortexer recommended)
- Water baths or heat blocks
- Centrifuge capable of centrifuging >12,000 × *g*
- Isopropanol
- 70% ethanol

Components supplied with the kit

- Cell Lysis Buffer (CLB)
- Proteinase K (lyophilized powder, see **Before Starting**, page 5)
- RNase A (4 mg/mL)
- Protein Precipitation Buffer (PPT)
- DNA Dissolving Buffer (TE)

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Purifying gDNA from Swabs, Continued

Preparing Swab Lysate

1. Collect the buccal swab with a suitable tool, such as a T-swab Kit, Dacron swab, C.E.P. Omni swab, or Cotton swab according to standard collection procedures. Nasal, pharyngeal, or vaginal swabs can be collected in a similar manner.
2. Set a water bath or heat block at 58°C.
3. Place the buccal swab into a capped 2 mL microcentrifuge tube (not provided with the kit).
   - For C.E.P. Omni swabs, press the stem end towards the swab to eject it into the microcentrifuge tube.
   - For swabs from other suppliers, snap or cut the swab at the break point.

   The swab should fit entirely inside the tube so that the cap may close.
4. Pipet 300 µL Cell Lysis Buffer (CLB) into a 1.5 mL reaction tube. Dip the collection brush into the CLB aliquot and rotate the swab 10–20 times to release as many cells as possible. Squeeze out the swab against the wall of the tube to recover the maximum amount of liquid.
5. Incubate at 58°C until the lysate is homogeneous and clear, vortexing the sample periodically to facilitate lysis.
6. *Optional:* Add 10 µL Proteinase K. Incubate at 58°C for 30 minutes to overnight until the mixture is clear, indicating complete lysis.
7. *Optional:* Add 10 µL RNase A. Incubate at 37°C for 5 minutes.
8. Allow the lysate to cool to room temperature and then proceed directly to **Purifying gDNA**, next page.

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Purifying gDNA from Swabs, Continued

Purifying gDNA

1. Add 150 µL Protein Precipitation Buffer (PPT) to 300 µL of swab lysate from Step 8, previous page.
2. Mix thoroughly by vortexing for 20 seconds until a homogenous suspension is obtained.
3. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or insignificant, proceed to **Procedure for Pellet Compacting**, page 38.
4. Transfer the supernatant into a clean, sterile microcentrifuge tube.
5. Add an equal volume of isopropanol. Mix thoroughly by inverting the tube until the mixture is homogenous. Do not vortex.
6. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated DNA is visible as a white pellet.
7. Decant the supernatant. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.
8. Add 1 mL 70% ethanol and wash the DNA pellet by inverting the tube several times.
9. Centrifuge at 12,000 × *g* for 1 minute at room temperature.
10. Carefully decant the supernatant.

    Note: Be careful not to discard the DNA as the DNA pellet may be loosely adherent to the tube.
11. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.

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Purifying gDNA from Swabs, Continued

Purifying gDNA, Continued

12. Evaporate residual ethanol by incubating the sample at 50 °C to 55°C for 10 minutes.

    Note: If desired, you may evaporate residual ethanol at room temperature, but in this case, increase the incubation time.

13. After ethanol evaporation is complete (i.e., DNA pellet appears dry and the ethanol odor is undetectable), resuspend the DNA pellet in a suitable volume of DNA Dissolving Buffer (TE).

14. Incubate the tube at room temperature overnight or at 65°C for up to one hour to completely resuspend the DNA.

    The DNA should be completely dissolved as a clear, colorless solution. If precipitates are present, the A_{260}/A_{280} ratio is <1.70, or the DNA is contaminated with buffer, protein, or RNA, re-purify the DNA sample (see **Purifying Impure DNA,** page 39).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Purifying gDNA from Bacteria

Introduction

The JetFlex Genomic DNA Purification Kits are designed to purify genomic DNA from bacterial cultures.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Materials Needed

- Sample for DNA isolation
- Lysozyme, 2 mg/mL, in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA buffer (for gram-positive bacteria only)
- Lysozyme, 2 mg/mL, and Lysostaphin, 1 mg/mL, in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA buffer (for *Staphylococcus* only)
- Sterile, DNase-free microcentrifuge tubes (for small preparations) or sterile, DNase-free 15 mL or 50 mL tubes (for larger sample preparation)
- Vortex mixer (multi-tube vortexer recommended)
- Water baths or heat blocks
- Centrifuge capable of centrifuging >12,000 × *g*
- Isopropanol
- 70% ethanol

Components supplied with the kit

- Cell Lysis Buffer (CLB)
- Proteinase K (lyophilized powder, see **Before Starting**, page 5)
- RNase A (4 mg/mL)
- Protein Precipitation Buffer (PPT)
- DNA Dissolving Buffer (TE)

Continued on next page

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Purifying gDNA from Bacteria, Continued

Preparing Bacterial Lysate

The following procedure is for purifying gDNA from a 500 µL culture sample. To purify DNA from a larger sample, scale up the buffer and reagent volumes proportionately.

1. Collect 500 µL of an overnight bacterial culture into a 1.5 mL microcentrifuge tube.
2. Centrifuge the sample at 12,000 × *g* for 1 minute. Remove the supernatant.
3. For gram-negative bacteria, proceed directly to Step 4.

   For gram-positive bacteria, disrupt cell walls with enzymatic pretreatment:

   a. Resuspend the cells in 300 µL 50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 2 mg/mL lysozyme.

      Note: For *Staphylococcus*, add 1 mg/mL lysostaphin to the 50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 2 mg/mL lysozyme buffer.

   b. Mix thoroughly and incubate for 30 minutes at 37°C.

   c. Centrifuge for 1 minute at 12,000 × *g* (or at 2,000 × *g* for 5 minutes, for cultures >500 µL).

   d. Remove the supernatant and proceed to Step 4..
4. Add 300 µL Cell Lysis Buffer (CLB) to the bacterial pellet.
5. Lyse the cells by pipetting up and down.

   Note: Usually no incubation is required. However, if cell clumps are visible after mixing, incubate at 58°C until the lysate is homogeneous and clear. Samples are stable in CLB for several months at room temperature.
6. *Optional:* Add 10 µL Proteinase K. Incubate at 58°C for 30 minutes to overnight until the mixture is clear, indicating complete lysis.
7. Add 10 µL RNase A. Incubate at 37°C for 5 minutes.
8. Allow the lysate to cool to room temperature and then proceed directly to **Purifying gDNA**, next page.

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Purifying gDNA from Bacteria, Continued

Purifying gDNA

1. Add 150 µL Protein Precipitation Buffer (PPT) to 300 µL of bacterial lysate from Step 8, previous page.
2. Mix thoroughly by vortexing for 20 seconds until a homogenous suspension is obtained.
3. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or insignificant, proceed to **Procedure for Pellet Compacting**, page 38.
4. Transfer the supernatant into a clean, sterile microcentrifuge tube.
5. Add an equal volume of isopropanol. Mix thoroughly by inverting the tube until the mixture is homogenous. Do not vortex.
6. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated DNA is visible as a white pellet.
7. Decant the supernatant. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.
8. Add 1 mL 70% ethanol and wash the DNA pellet by inverting the tube several times.
9. Centrifuge at 12,000 × *g* for 1 minute at room temperature.
10. Carefully decant the supernatant.

    Note: Be careful not to discard the DNA as the DNA pellet may be loosely adherent to the tube.
11. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.

Continued on next page

Purifying gDNA from Bacteria, Continued

Purifying gDNA, Continued

12. Evaporate residual ethanol by incubating the sample at 50 °C to 55°C for 10 minutes.

    Note: If desired, you may evaporate residual ethanol at room temperature, but in this case, increase the incubation time.

13. After ethanol evaporation is complete (i.e., DNA pellet appears dry and the ethanol odor is undetectable), resuspend the DNA pellet in a suitable volume of DNA Dissolving Buffer (TE).

14. Incubate the tube at room temperature overnight or at 65°C for up to one hour to completely resuspend the DNA.

    The DNA should be completely dissolved as a clear, colorless solution. If precipitates are present, the A_{260}/A_{280} ratio is <1.70, or the DNA is contaminated with buffer, protein, or RNA, re-purify the DNA sample (see **Purifying Impure DNA,** page 39).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Purifying gDNA from Yeast

Introduction

The JetFlex Genomic DNA Purification Kits are designed to purify genomic DNA from yeast cultures.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Materials Needed

- Sample for DNA isolation
- Buffer YS1, see below for recipe
- Zymolyase
- Sterile, DNase-free microcentrifuge tubes (for small preparations) or sterile, DNase-free 15 mL or 50 mL tubes (for larger sample preparation)
- Vortex mixer (multi-tube vortexer recommended)
- Water baths or heat blocks
- Centrifuge capable of centrifuging >12,000 × *g*
- Isopropanol
- 70% ethanol

Components supplied with the kit

- Cell Lysis Buffer (CLB)
- Proteinase K (lyophilized powder, see **Before Starting**, page 5)
- RNase A (4 mg/mL)
- Protein Precipitation Buffer (PPT)
- DNA Dissolving Buffer (TE)

Buffer YS1

Prepare Buffer YS1:

0.9 M sorbitol
0.1 M EDTA, pH 7.5
14 mM β-mercaptoethanol

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Purifying gDNA from Yeast, Continued

Prepare Zymolyase

Preparing the cell lysate includes an incubation step with Zymolyase (see Step 5, below). Zymolyase digests cell walls of yeast cells enzymatically during incubation. An equivalent enzyme to Zymolyase is Lyticase (Sigma, Cat. No. L2524). Dilute Zymolyase or Lyticase in distilled water to a final concentration of 1,000 U/mL. Store stock solutions of both enzymes in aliquots at –20°C and use only once.

Preparing Yeast Cell Lysate

The following procedure is for purifying gDNA from a 1 mL culture sample. To purify DNA from a larger sample, scale up the buffer and reagent volumes proportionately.

1. Grow the Saccharomyces (or any other yeast) culture to saturation in YPD or YEPD to a final concentration of 1–2 × 10^8 cells/mL.
2. Harvest 1 mL of the yeast culture by centrifugation at 12,000 × *g* for 1 minute. Discard the supernatant.

   Note: For >1 mL – 10 mL yeast culture samples, centrifuge at 2,000 × *g* for 3 minutes.
3. Wash the cells in 300 µL Buffer YS1.
4. Centrifuge again at 12,000 × *g* for 1 minute. Discard the supernatant. Resuspend the cells in 1 mL Buffer YS1.
5. Add 10 µL Zymolyase or Lyticase (1,000 U/mL) and incubate at 37°C for 30 minutes.

   Note: Monitor spheroplast formation by examining detergent sensitivity: dilute a small sample of cells in 1% SDS. Spheroplasting is sufficient when greater than 90% of the cells burst when examined under the microscope.
6. Centrifuge the spheroplasts at 12,000 × *g* for 1 minute. Discard the supernatant.
7. Add 300 µL Cell Lysis Buffer (CLB) to the yeast pellet.

Continued on next page

Purifying gDNA from Yeast, Continued

Preparing Lysate, Continued

8. Lyse the spheroplasts by pipetting up and down.

   Note: Usually no incubation is required. However, if cell clumps are visible after mixing, incubate at 58°C until the lysate is homogeneous and clear. Samples are stable in CLB for several months at room temperature.

9. Add 10 µL RNase A. Incubate at 37°C for 5 minutes.
10. Allow the lysate to cool to room temperature and then proceed directly to **Purifying gDNA**, below.

Purifying gDNA

1. Add 150 µL Protein Precipitation Buffer (PPT) to 300 µL of yeast cell lysate from Step 10, above.
2. Mix thoroughly by vortexing for 20 seconds until a homogenous suspension is obtained.
3. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or insignificant, proceed to **Procedure for Pellet Compacting,** page 38.

4. Transfer the supernatant into a clean, sterile microcentrifuge tube.
5. Add an equal volume of isopropanol. Mix thoroughly by inverting the tube until the mixture is homogenous. Do not vortex.
6. Centrifuge at 12,000 × *g* for 3 minutes at room temperature.

   Note: The precipitated DNA is visible as a white pellet.

7. Decant the supernatant. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.

Continued on next page

Purifying gDNA from Yeast, Continued

Purifying gDNA, Continued

8. Add 1 mL 70% ethanol and wash the DNA pellet by inverting the tube several times.
9. Centrifuge at 12,000 × *g* for 1 minute at room temperature.
10. Carefully decant the supernatant.

    Note: Be careful not to discard the DNA as the DNA pellet may be loosely adherent to the tube.
11. Allow residual liquid to drain on a sheet of absorbent paper towel for a few minutes.
12. Evaporate residual ethanol by incubating the sample at 50 °C to 55°C for 10 minutes.

    Note: If desired, you may evaporate residual ethanol at room temperature, but in this case, increase the incubation time.
13. After ethanol evaporation is complete (i.e., DNA pellet appears dry and the ethanol odor is undetectable), resuspend the DNA pellet in a suitable volume of DNA Dissolving Buffer (TE).
14. Incubate the tube at room temperature overnight or at 65°C for up to one hour to completely resuspend the DNA.

    The DNA should be completely dissolved as a clear, colorless solution. If precipitates are present, the A_{260}/A_{280} ratio is <1.70, or the DNA is contaminated with buffer, protein, or RNA, re-purify the DNA sample (see **Purifying Impure DNA,** page 39).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Estimating DNA Yield

DNA Yield

Perform DNA quantitation using UV absorbance at 260 nm or Quant-iT™ DNA Assay Kits (see page 40 for ordering information).

UV Absorbance

1. Prepare a dilution of the DNA solution in 10 mM Tris-HCl, pH 7.5. Mix well. Measure the absorbance of the dilution at 260 nm (A_{260}) in a spectrophotometer (using a cuvette with an optical path length of 1 cm) blanked against 10 mM Tris-HCl pH 7.5.
2. Calculate the concentration of DNA using the formula:

   DNA (µg/mL) = $A_{260} \times 50 \times$ dilution factor

For DNA, A_{260} = 1 for a 50 µg/mL solution measured in a cuvette with an optical path length of 1 cm.

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits (page 40) provide a rapid, sensitive, and specific method for dsDNA quantitation with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance.

The kit contains a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a pre-made buffer. The assay is designed for reading in standard fluorescent readers/fluorometer or Qubit® Fluorometer.

Troubleshooting

Introduction

Refer to the table below to troubleshoot problems that you may encounter when using the JetFlex Genomic DNA Purification Kits.

Problem	Cause	Solution
Low DNA yield	Starting blood or body fluid sample too dilute	Concentrate samples by centrifugation at 2,000 × *g* for 10 minutes prior to processing
	Poor quality of starting material	Use fresh samples and process immediately after collection or freeze the samples at –80°C or in liquid nitrogen. The yield and quality of DNA isolated is dependent on the type and age of the starting material.
	Incorrect lysis conditions	For blood samples, use a 1:1 ratio of sample:Red Blood Cell Lysis Buffer. For all samples, use the recommended volume of Cell Lysis Buffer, as directed in the protocol.
	Low starting DNA content	If the DNA yield is expected to be low, use a carrier (i.e., 10 µg Glycogen per 300 µL isopropanol) to enhance DNA precipitation.
DNA contaminated with protein	Protein not precipitated during protein precipitation step, indicated by a loose or small protein pellet	Precipitate the protein with the **Procedure for Pellet Compacting**, page 38

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
Low DNA yield	DNA is sheared or degraded	Avoid repeated freezing and thawing of samples to prevent any DNA damage. Maintain a sterile environment while working to avoid any contamination from DNases.
Dark colored eluate (mammalian tissue, mouse tails, or blood samples only)	Pigments from tissues or heme from blood co-precipitate with DNA	• Use RBC Buffer at room temperature. Chilled RBC Buffer may prevent lysis. • Purify DNA using **Purifying Impure DNA**, page 39.
Blood sample is coagulated or contains clots	Frozen blood samples thawed too slowly	• If the blood sample contains clots or is coagulated, mince the sample with a pestle. • Thaw frozen blood samples quickly at 37°C to minimize clotting, white blood cell lysis and DNase activity. • Purify DNA from the unclotted portion of the sample.
Impure DNA: $A_{260}/A_{280} < 1.70$ or insoluble particles present with dissolved DNA	Excess starting material	Purify DNA using **Purifying Impure DNA**, page 39.

Purifying Impure DNA

Introduction

The procedure described below may be used to re-purify DNA when the redissolved DNA contains insoluble particles, is discolored, or is impure or contaminated. The procedure is based on a 100 µL starting sample volume, but may be scaled up or down proportionately.

Materials Needed

- Purified DNA sample
- Centrifuge capable of centrifuging >12,000 × *g*
- Isopropanol
- 70% ethanol

Components supplied with the kit

- Cell Lysis Buffer (CLB)
- Protein Precipitation Buffer (PPT)
- Pellet Compactor
- DNA Dissolving Buffer (TE)

Repurifying DNA

1. Add 500 µL Cell Lysis Buffer for every 100 µL purified DNA sample. Mix thoroughly by inverting tube or pipetting up and down.

   Note: If DNA sample contains particulates, incubate at 37°C. Cool sample to room temperature before proceeding.
2. Add 300 µL Protein Precipitation Buffer. Vortex vigorously for 20 seconds to homogenize the mixture.
3. Centrifuge at 12,000 × *g* for 3 minutes to pellet any precipitated impurities.

   Note: If a pellet is not visible, proceed to **Pellet Compacting**, previous page, making certain to use the appropriate amount of Pellet Compactor based on your sample volume.
4. Proceed to Step 4, **Purifying gDNA**, for the specific sample type (i.e., page 9, for blood).

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Accessory Products

Additional Products

The table below lists additional products available from Genomed or Invitrogen that may be used with the JetFlex Genomic DNA Purification Kit.

Product	Amount	Cat. no.
Products below are available from Genomed. See www.genomed-dna.com		
Red Blood Cell Buffer (RBC)	200 mL	RBC-200
Cell Lysis Buffer (CLB)	125 mL	CLB-125
Protein Precipitation Buffer (PPT)	50 mL	PPT-050
DNA Dissolving Buffer (TE)	100 mL	TE-100
Proteinase K	40 mg	GN-PK-040
	200 mg	GN-PK-200
Ribonuclease A	50 mg	GN-RN-50
	100 mg	GN-RN-100
Products below are available from Invitrogen. See www.invitrogen.com		
Phosphate Buffered Saline (PBS), 1X	500 mL	10010-023
Luria Broth Base (Miller's LB Broth Base), powder	500 g	12795-027
	2.5 kg	12795-084
Ampicillin Sodium Salt, irradiated	200 mg	11593-027
Carbenicillin, Disodium Salt	5 g	10177-012
Quant-iT™ DNA Assay Kit, High Sensitivity	1,000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1,000 assays	Q33130
Qubit® Fluorometer	1 each	Q32857

Purchaser Notification

Limited Warranty

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