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JetQuick™ Genomic DNA Purification Kits

USER GUIDE

For the purification of genomic DNA from blood, body fluids, and mammalian cells

Catalog Numbers A30703, A30704, A30705, and A30706

Publication Number MAN0001742

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A.0	25 April 2016	Updated to current branding guidelines and standards
—	12 August 2010	Baseline for revisions

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Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

JetQuick™ Genomic DNA Purification Kits are designed for rapid and efficient purification of genomic DNA (gDNA) from blood, body fluids (amniotic fluid, saliva, sperm, lymph...), and mammalian cells. Their silica membrane-based technology allows for isolation of DNA of 20–50 kb in size and that is suitable for a wide range of downstream applications, including PCR, restriction enzyme digestion, and Southern blotting.

Contents and storage

Table 1 JetQuick™ Blood and Cell Culture DNA Midiprep Kit (Cat. Nos. A30703 and A30704)

Contents	Cat. No. A30703 (20 preps)	Cat. No. A30704 (50 preps)	Storage
Buffer K1	66 mL	165 mL	15 to 30°C
Buffer K2	72 mL	2 × 87 mL	
Buffer KX	105 mL	2 × 124 mL	
Elution Buffer (10 mM Tris-HCl, pH 8.5)	44 mL	88 mL	
RNase A (20 mg/mL)	2 × 1.3 mL	4 × 1.3 mL	2°C to 8°C[1]
Protease (lyophilized powder)	5 × 26 mg	320 mg	
JetQuick™ Spin Columns (5 columns per bag)	4 bags	10 bags	15 to 30°C

[1] For long-term storage, store in single-use aliquots at –25°C to –15°C.

Table 2 JetQuick™ Blood and Cell Culture DNA Maxiprep Kit (Cat. Nos. A30705 and A30706)

Contents	Cat. No. A30705 (20 preps)	Cat. No. A30706 (50 preps)	Storage
Buffer K1	220 mL	550 mL	15 to 30°C
Buffer K2	72 mL	2 × 87 mL	
Buffer KX	105 mL	2 × 124 mL	
Elution Buffer (10 mM Tris-HCl, pH 8.5)	44 mL	110 mL	
RNase A (20 mg/mL)	5 × 1.3 mL	13 × 1.3 mL	2°C to 8°C[1]
Protease (lyophilized powder)	220 mg	560 mg	
JetQuick™ Spin Columns (5 columns per bag)	4 bags	10 bags	15 to 30°C

[1] For long-term storage, store in single-use aliquots at –25°C to –15°C.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Item	Source
Equipment	
Benchtop microcentrifuge	MLS
Centrifuge with a swinging bucket rotor capable of reaching >12,000 × *g* (for isolation using centrifugation only)	MLS
EveryPrep™ Universal Vacuum Manifold (for isolation using vacuum manifold only)	K211101
Laboratory mixer (Vortex or equivalent)	MLS
Water bath or heat block at 70°C	MLS
Tubes, plates, and accessories	
Disposable, individually wrapped, sterile plasticware	MLS
Sterile, DNase-free tubes, 50 mL	MLS
Automatic pipetors	MLS
Aerosol-resistant pipette tips	MLS

Item	Source
Reagents	
Ethanol, 95–100%	10010–023
Phosphate Buffered Saline (PBS), 1X	MLS
TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)	MLS
(Optional) Tris_HCl, pH 8.5	MLS
(Optional) $MgCl_2$	MLS
(Optional) Sucrose	MLS
(Optional) Triton™ X-100	MLS

Methods

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Use disposable, individually wrapped, sterile plasticware.
- Use only sterile, new pipette tips and microcentrifuge tubes.
- Maintain a sterile environment when handling DNA to avoid any contamination from DNases.
- Do not vortex samples for more than 5–10 seconds at each vortexing step to avoid extensive shearing of gDNA.
- Loosely attach a cap to the Receiver Tubes to allow ventilation during centrifugation.
- To minimize DNA degradation, perfom lysate preparation steps quickly and avoid repeated freezing and thawing of the samples.
- Wear a laboratory coat, disposable gloves, and eye protection when handling blood samples.

Before you begin

Before first use of the kit

- Reconstitute Buffer K2 and Buffer KX with 100% ethanol as instructed on the labels, mix well, then store at room temperature.
- Resuspend Protease in double-distilled water to a final concentration of 20 mg/ml, then store the reconstituted enzyme in single-use aliquots at –20°C.
- Prepare White Blood Cell (WBC) Buffer (10 mM Tris-HCl, pH 8.5; 5 mM $MgCl_2$; 320 mM sucrose; 1% Triton™ X-100) if you use blood samples >10 mL.

Before each use of the kit

- Heat water bath or heat block at 70°C.
- Prewarm an appropriate volume of Elution Buffer at 70°C.

Purify gDNA from blood and body fluids using centrifugation

(Optional) Prepare concentrated lysates

If you are using blood samples >10 mL, follow the following procedure to concentrate the samples. Otherwise, proceed directly to "Lyse the samples" on page 9.

1. Place the whole blood sample into a sterile 50-mL harvesting tube.
2. Add an equal volume of WBC Buffer to the sample.
3. Mix thoroughly by inverting the tube several times.
4. Centrifuge for 2 minutes at 5000 × *g*.
5. Aspirate the supernatant with a pipette.
   Do not disturb the light-red white blood cell pellet.
6. Resuspend the pellet in 9.5 mL of PBS 1X.
7. Mix by pulse-vortexing.

Proceed immediately to "Lyse the samples" on page 9

Lyse the samples

1. Transfer the sample to a clean, sterile 50-mL harvesting tube.

Size	Volume
Midiprep	3 mL
Maxiprep	10 mL

2. Add the following volume of Protease to the sample, then mix well by vortexing.

Size	Volume
Midiprep	300 µL
Maxiprep	500 µL

3. *(Optional)* Add the following volume of RNase A, then mix well by vortexing.

Size	Volume
Midiprep	100 µL
Maxiprep	300 µL

4. Add the following volume of Buffer K1, then mix well by vortexing.

Size	Volume
Midiprep	3 mL
Maxiprep	10 mL

5. Incubate for 10 minutes at 70°C to degrade the proteins.

6. Add the following volume of ethanol, then mix well by vortexing.

Size	Volume
Midiprep	3 mL
Maxiprep	10 mL

IMPORTANT! Mix the sample immediately to prevent nucleic acid precipitation due to high local alcohol concentration.

Bind the DNA to the membrane

Place a JetQuick™ Spin Column in a 50-mL Receiver tube.

1. Apply the sample to the JetQuick™ Spin Column.
2. Centrifuge for 3 minutes at 2000 × *g*.
3. Discard the flow-through.

Wash the DNA on the membrane

1. Add 10 mL of Buffer KX.
2. Centrifuge for 2 minutes at 5000 × *g*.
3. Discard the flow-through.
4. Add 10 mL of Buffer K2.
5. Centrifuge for 2 minutes at 5000 × *g*.
6. Discard the flow-through.
7. Centrifuge for 10 minutes at 5000 × *g* to remove any residual liquid.
8. Discard the Receiver Tube.

Elute the DNA

1. Place the JetQuick™ Spin Column on a clean 50-mL Elution Tube.
2. Add prewarmed Elution Buffer according to the following table.

Size	Volume
Midiprep	500–800 µL
Maxiprep	1–4 mL

3. Incubate for 5 minutes at room temperature.
4. Centrifuge for 2 minutes at 5000 × *g*.
5. *(Optional)* Perform a second elution in another Elution Tube to recover more DNA

The Elution Tube contains the purified DNA.

Store the purified DNA:

- at 4°C for immediate use.
- at –20°C in aliquots for longer-term storage.

Purify gDNA from blood and body fluids using vacuum

Lyse the samples

1. Transfer the sample to a clean, sterile 50-mL harvesting tube.

Size	Volume
Midiprep	3 mL
Maxiprep	10 mL

2. Add the following volume of Protease to the sample, then mix well by vortexing.

Size	Volume
Midiprep	300 µL
Maxiprep	500 µL

3. *(Optional)* Add the following volume of RNase A, then mix well by vortexing.

Size	Volume
Midiprep	100 µL
Maxiprep	300 µL

4. Add the following volume of Buffer K1, then mix well by vortexing.

Size	Volume
Midiprep	3 mL
Maxiprep	10 mL

5. Incubate for 10 minutes at 70°C to degrade the proteins.

6. Add the following volume of ethanol, then mix well by vortexing.

Size	Volume
Midiprep	3 mL
Maxiprep	10 mL

IMPORTANT! Mix the sample immediately to prevent nucleic acid precipitation due to high local alcohol concentration.

Bind the DNA to the membrane

Attach vacuum manifold to a vacuum source. Attach JetQuick™ Spin Column to the vacuum manifold.

1. Apply the sample to the JetQuick™ Spin Column.
2. Apply vacuum (-200 to -650 mbar) until all liquid is pulled through the column.
3. Turn off the vacuum source.

Wash the DNA on the membrane

1. Add 10 mL of Buffer KX.
2. Apply vacuum (-200 to -650 mbar) until all liquid is pulled through the column.
3. Turn off the vacuum source.
4. Add 10 mL of Buffer K2.
5. Apply vacuum (-200 to -650 mbar) until all liquid is pulled through the column.
6. Turn off the vacuum source.
7. Apply vacuum (-200 to -650 mbar) to remove any residual liquid.
8. Turn off the vacuum source.

Elute the DNA

1. Place the JetQuick™ Spin Column on a clean 50-mL Elution Tube.
2. Add prewarmed Elution Buffer according to the following table.

Size	Volume
Midiprep	500–800 µL
Maxiprep	1–4 mL

3. Incubate for 5 minutes at room temperature.
4. Centrifuge for 2 minutes at 5000 × *g*.
5. *(Optional)* Perform a second elution in another Elution Tube to recover more DNA

The Elution Tube contains the purified DNA.

Store the purified DNA:

- at 4°C for immediate use.
- at –20°C in aliquots for longer-term storage.

Purify gDNA from mammalian cells

The following table lists the amount of starting material recommended for each purification kit.

Size	Number of cells
Midiprep	7.5×10^7 to 1×10^8
Maxiprep	2.5×10^8 to 5×10^8

Prepare the cells

1. Harvest the cells in a 50-mL harvesting tube.
   - **For cells grown in monolayer:** remove the growth medium for the culture plate and harvest the cells by trypsinization or use a cell scraper according to established protocols.
   - **For cells grown in suspension:** collect the cells directly in the harvesting tube.
2. Centrifuge for 5 minutes at 300–350 × *g* to pellet the cells.
3. Discard the supernatant.
4. Resuspend the pellet in 1X PBS according to the following table.

Size	Volume
Midiprep	3 mL
Maxiprep	10 mL

Lyse the samples

1. Transfer the sample to a clean, sterile 50-mL harvesting tube.

Size	Volume
Midiprep	3 mL
Maxiprep	10 mL

2. Add the following volume of Protease to the sample, then mix well by vortexing.

Size	Volume
Midiprep	300 µL
Maxiprep	500 µL

3. *(Optional)* Add the following volume of RNase A, then mix well by vortexing.

Size	Volume
Midiprep	100 µL
Maxiprep	300 µL

4. Add the following volume of Buffer K1, then mix well by vortexing.

Size	Volume
Midiprep	3 mL
Maxiprep	10 mL

5. Incubate for 10 minutes at 70°C to degrade the proteins.

6. Add the following volume of ethanol, then mix well by vortexing.

Size	Volume
Midiprep	3 mL
Maxiprep	10 mL

IMPORTANT! Mix the sample immediately to prevent nucleic acid precipitation due to high local alcohol concentration.

Bind the DNA to the membrane

Place a JetQuick™ Spin Column in a 50-mL Receiver tube.

1. Apply the sample to the JetQuick™ Spin Column.
2. Centrifuge for 3 minutes at 2000 × *g*.
3. Discard the flow-through.

Wash the DNA on the membrane

1. Add 10 mL of Buffer KX.
2. Centrifuge for 2 minutes at 5000 × *g*.
3. Discard the flow-through.
4. Add 10 mL of Buffer K2.
5. Centrifuge for 2 minutes at 5000 × *g*.
6. Discard the flow-through.
7. Centrifuge for 10 minutes at 5000 × *g* to remove any residual liquid.
8. Discard the Receiver Tube.

Elute the DNA

1. Place the JetQuick™ Spin Column on a clean 50-mL Elution Tube.
2. Add prewarmed Elution Buffer according to the following table.

Size	Volume
Midiprep	500–800 µL
Maxiprep	1–4 mL

3. Incubate for 5 minutes at room temperature.

4. Centrifuge for 2 minutes at 5000 × *g*.

5. *(Optional)* Perform a second elution in another Elution Tube to recover more DNA

The Elution Tube contains the purified DNA.

Store the purified DNA:

- at 4°C for immediate use.
- at –20°C in aliquots for longer-term storage.

A Troubleshooting

Observation	Possible cause	Recommended action
The DNA yield is low	The lysis was incomplete.	Decrease the amount of starting material used.
		Add Protease during lysis.
		Increase the digestion time or amount of Protease used for lysis.
	The starting material is of poor quality.	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 is dependent on the type and age of the starting material.
	The binding conditions were incorrect.	Add 96–100% ethanol to the lysate prior to loading the samples on the spin column. Mix the samples by vortexing.
		Avoid overloading the column.
	Ethanol was not added to Buffers KX and K2.	Reconstitute Buffers KX and K2 according to "Before first use of the kit" on page 8.
	The elution conditions were incorrect.	Add Elution Buffer and perform incubation with buffer before centrifugation.
		Perform a second elution step.
	DNA is sheared or degraded.	Avoid repeated freezing and thawing of samples.
		Maintain a sterile environment to avoid DNases contamination.
The eluate is dark in color or the membrane is discolored	Pigments from tissues or heme from blood bind to the silica matrix and co-elute with DNA.	Be sure to add ethanol to the lysate prior to loading onto the spin columns. The ethanol prevents the pigments from sticking onto the silica matrix.
		Centrifuge the lysate at higher speed and longer prior to loading the lysate onto the spin columns.
The gDNA is contaminated by RNA	The membrane bound total nucleic acid present in the sample.	Perform a RNase A digestion step during the sample preparation.

Observation	Possible cause	Recommended action
Downstream enzymatic reactions are inhibited	Ethanol is present in the purified DNA.	1. Discard Wash Buffer flow-through. 2. Centrifuge the spin column at maximum speed for up to 15 minutes or incubate the spin column for 10 minutes at 70°C in an incubator to remove residual ethanol.
	Salts are present in the purified DNA.	Perform washes in the correct order.
		Maintain a ratio of 1:1:1 for sample:Buffer K1:Ethanol.
The elution volume is or the samples are cross-contaminated	The vacuum pressure is incorrect.	Make sure the vacuum manifold is tightly sealed without leakage.
		Maintain the vacuum pressure at –6 to –12 inches Hg (–200 to –400 mbar or –150 to –300 mm Hg).

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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  - 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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