Biomiga, Inc.

The Inventor of EZgene™ Plasmid Purification System

Genomic DNA Isolation Kit for

- Blood
- Tissue
- Mouse tail
- Paraffin-embedded tissue

For *in vitro* research use only

If crystals form in buffers, warm up at 37°C to dissolve before use

Contents

Introduction

The EZgene™ blood gDNA kit simplifies the purification of nucleic acids from blood samples. The system utilizes the reversible nucleic acid-binding properties of our ezBind membrane and the speed and versatility of spin column technology to yield high quality gDNA with the $OD_{260/280}$ ratio of 1.8-2.0. Up to 250 µL of fresh, frozen or anticoagulated whole blood can be readily processed at one time. This DNA Kit can also be used for the preparation of genomic DNA from buffy coat, serum, plasma, saliva, buccal swab and other body fluids. The EZgene™ Blood DNA Kit allows for the single or multiple simultaneous processing of samples. Purified DNA is ready for applications such as PCR, Southern Blotting, and Restriction Digestion.

Benefits of the EZgene™ Blood DNA Kit

Reliability-Optimized buffers that guarantee pure DNA
Safety-No organic extractions P
Quality-Purified DNA can be directly used for most downstream applications

Storage and Stability

All EZgene™ Tissue DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: reconstituted Proteinase K -20°C, and all other materials at RT (22-25°C).

Binding Capacity

Each ezBind™ DNA mini column can bind approximately 100 µg DNA. Using greater than 30 mg tissue or $1x10^7$ cells is not recommended.

Kit Contents

Buffer TL: For tissue genomic DNA kit.

Catalog#	GD2311-01	GD2311-02	GD2311-00
Preps	50	250	4
DNA Mini Columns	50	250	4
Buffer TL*	20 ml	60 ml	2ml
Buffer BL	20 ml	60 ml	2ml
Buffer KB	30 ml	110 ml	2ml
DNA Wash Buffer	12 ml	50 ml	1.25ml
Elution Buffer	30 ml	80 ml	500µl
RNase A	110 µL	500 µL	10 µL
Proteinase K	1.5 mL	6 mL	150 µL
User Menu	1	1	1

CAUTION! Buffer BL contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste. Use gloves and protective eyeware when handling this solution.

Before Starting

It is strongly advised that you familiarize yourself with the entire booklet before starting.
EZgene™ Kits are designed to be simple, fast, and reliable provided that all steps are followed diligently.

Materials and Reagents to be supplied by user

- Tabletop microcentrifuge
- Sterile 1.5mL centrifuge tubes
- Water bath
- RNase stock solution
- Absolute ethanol
- PBS Buffer

Important Notes:

- Dilute DNA Wash Buffer with absolute ethanol as follows:
  GD2311-01: Add 8 ml absolute ethanol (96-100%) to each bottle
  GD2311-01: Add 48 ml absolute ethanol (96-100%) to each bottle
  GD2311-02: Add 200 ml absolute ethanol (96-100%) to each bottle

- Preheat Elution Buffer to 65° C (in aliquots of 0.5 ml/ sample)

- For *Mouse Tail Protocol* have the following ready: Pre-mixed solutions of 200 µl of Buffer BL and 210µl of absolute ethanol (per sample). Mix by vortexing. They can be prepared fresh, or pre-made and stored at room temperature. Do not store this mixture for more than one month

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl Buffer, or Elution Buffer as a blank. DNA concentration is calculated as:

[DNA] = (Absorbance$_{260}$) x (0.05 µg/µl) x (Dilution Factor)

The quality of DNA can be assessed by measuring absorbance at both 260 nm and 280 nm. An A_{260}/A_{280} ratio of 1.7-1.9 corresponds to 85%-95% purity.

Expected yields vary with both amount, and type of tissue used; 30 mg of fresh tissue will yield 10 -40 µg of DNA with two elutions (each 200 µl).

Concentrate DNA

If necessary the DNA can be concentrated. Add sodium chloride to reach a final concentration of 0.1 M followed by 2x volume of absolute (~96-100%) ethanol. Mix well and incubate at -20°C for 10 min. Centrifuge at 10,000 x g for 15 min and discard supernatant. Add 700µl of 70% ethanol and centrifuge at 10,000 x g for 2 min. Discard supernatant, air dry the pellet for 2 min, and resuspend the DNA in 20 µl of sterile deionized water or 10 mM Tris-HCl, pH 8.5.

A. EZgene™ Tissue Genomic DNA Purification Protocol

This method is suitable for the isolation of genomic DNA from up to 30 mg of tissue. Yields vary depending on source.

OPTIONAL: Although no mechanical homogenization of tissue is necessary, pulverizing the samples in liquid nitrogen will improve lysis and reduce incubation time. Once the liquid nitrogen has evaporated, transfer the powdered tissue into a clean 1.5 ml tube. Add 200 µl of Buffer TL and proceed to step 2 below.

1. Mince up to 20-30 mg tissue and place it into a 1.5 ml microtube. Add 200 µl Buffer TL. In order to speed up lysis, cut the tissue into small pieces. For samples more than 30 mg, simply scale up the volume of Buffer TL used; for a 40-60 mg sample use 400 µl of Buffer TL.

2. Add 25 µl reconstituted Proteinase K. Vortex to mix, and incubate in a shaking water bath set to 55°C to effectively complete lysis. If no shaking water-bath is available, vortex the sample every 20-30 min. Lysis time depends on the amount and type of tissue used; average time is usually under 3 hours. One can allow lysis to proceed overnight.

3. OPTIONAL: Certain tissues such as liver tissue have high levels of RNA, which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. To do so, add 4 µl (assuming a sample size of 30 mg) RNase A (100 mg/ml) and incubate at room temperature for 2 min. Proceed with the tissue protocol.

4. Centrifuge for 5 min at ≥13,000 x g to pellet insoluble tissue debris. CAREFULLY aspirate the supernatant and transfer to a sterile microtube.

5. Add 220 µl Buffer BL. Vortex to mix. Incubate at 70°C for 10 min.
   A wispy precipitate may form upon addition of Buffer BL, but does not interfere with DNA recovery. Adjust the volume of Buffer BL required based on the amount of starting material.

6. Add 220 µl Absolute Ethanol (96-100%, room temperature). Vortex to mix.
   Adjust the volume of ethanol required based on the amount of starting material.

7. Transfer the entire sample from step 6 into the column including any precipitate that may have formed. Centrifuge at 10,000 x g for 1 min. Discard the flow-through liquid.

8. Place the DNA Mini Column into the collection tube and add 500 µl Buffer KB. Centrifuge at 10,000 x g for 1 min. Discard the flow-through liquid.

9. Place the DNA Mini Column into the collection tube. Add 500 µl DNA Wash Buffer. Centrifuge as above and discard flow-through liquid.
   Ensure that absolute ethanol has been added to the DNA Wash Buffer before use. See label for directions. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.

10. Using the same 2 ml collection tube, repeat step 9.

11. Using the same 2 ml collection tube, and centrifuge with the lid open at maximum speed (≥13,000 x g) for 2 min to remove the residual ethanol.
    This step is crucial for ensuring optimal elution.

12. Place the DNA Mini Column into a sterile 1.5 ml microtube, and add 100-200 µl preheated (65°C) Elution Buffer (10mM Tris, pH8.5). Allow to sit at room temperature for 1-3 min. Centrifuge at ≥13,000 x g for 1 min.

13. Optional: Repeat the elution with a second 100-200µl Elution Buffer.

    Each 200µl elution typically produces yields of 60-70% of the DNA bound to the column. Thus two elutions will generally give ~90% yields. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µl of Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µl greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than at room temperature) upon the addition of Elution Buffer.

B. EZgene™ Blood Genomic DNA Purification Protocol

NOTE: The procedure below has been optimized for the use with fresh or frozen blood samples of 1~100 µL in volume. Anticoagulated Blood, Saliva, Serum, Buffy Coat or other Body Fluids can also be used. In addition, 10^7 of leukocytes or cultured cells may be used with this procedure.

For DNA extraction from tissue and mouse tail we suggest that you use the Tissue DNA Kit procedure. To isolate Viral RNA from serum or other non-cellular body fluids we recommend using the EZgene™ Viral RNA Kit.

1. Add 25 µL of Reconstituted Protease K to a clean 1.5 mL tube. Transfer 50-100 µL anticoagulated blood to the tube. Bring the volume up to 200 µL with 10 mM Tris-HCl, pH 8.0, PBS, or Elution Buffer (provided).

2. Add and 200 µL of Buffer BL. Mix thoroughly by vortexing at maximum speed for 10 seconds. If RNA-Free Genomic DNA is required, add 2 µL of RNase A (100 mg/mL) to each sample.

3. Incubate the sample at 50 ℃ for 10 minutes. Briefly vortex the tube once during incubation.

4. Add 200 µL of absolute ethanol (RT, 96-100%) to the lysate. Vortex at maximum speed for 10 seconds. Briefly centrifuge the tube to collect any drops from the inside of the lid.

5. Transfer the sample from step 4 into the column, and centrifuge at 10,000 x g for 30 seconds. Discard the collection tube and flow-through liquid.

6. Place the column into a new provided 2 mL collection tube. Add 500 µL of Buffer KB, and centrifuge at maximum speed for 30 seconds. Discard the collection tube and flow through liquid.

7. Place the column into the same 2 mL collection tube from step 6, and add 600 µL of DNA Wash Buffer. Centrifuge at 10,000 x g for 30 seconds. Discard the collection tube and flow-through liquid.

8. Place the column into the same 2 mL collection tube, and add 600 µL of DNA Wash Buffer. Centrifuge at 10,000 x g for 1 min. Discard the flow-through liquid.

NOTE: DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle, and on page 3. If refrigerated, the diluted DNA Wash Buffer must be brought to room temperature before use.

9. Place the empty column into the collection tube and centrifuge at 13,000 x g for 1 min to dry the column.
Note: This step removes residue ethanol.

10. Transfer the column into a sterile 1.5 mL microtube, add 75 - 100 µL preheated (65℃) Elution Buffer (10mM Tris-HCl, pH 8.5). Allow sit at room temperature for 2 - 5 minutes.

11. Centrifuge at 13,000 x g for 1 min to elute the DNA. The first elution normally yield 60-70% of DNA bound.

12. Option: Add the eluted DNA back to the column and centrifuge at 13,000 x g for 1 min. The second elution will yield another 20-30% of the DNA bound.

C. EZgene™ Mouse Tail Genomic DNA Purification Protocol

Bring frozen samples and Proteinase K solution to room temperature, and pre-heat aliquots of Elution Buffer (0.5 ml / sample) at 70°C.

1. Snip two pieces of mouse tail 0.2 - 0.5 cm in length. Place into a sterile 1.5 ml microcentrifuge tube, and add 180 µl Buffer TL. If necessary cauterize the wound to stop bleeding. Having appropriately earmarked the animal, return it to a clean cage. Mice should not be older than 6 weeks, since lysis will be more difficult which results in suboptimal DNA yields. If possible, obtain a tail biopsy at 2-4 weeks and freeze samples at -70°C until DNA is extracted.

2. Add 25 µl reconstituted Proteinase K. Vortex to mix well, and incubate in a shaking water bath set to 55°C for 1-4 hours or until lysis is complete. If no shaking water-bath is available, vortex the sample every 20-30 minutes.
Incomplete lysis may block column flow and significantly reduce DNA yields. Incubation time for complete tail lysis is dependent on tail length, and animal age; 0.5 cm tail pieces from a 2-week old mice typically lyses in approximately 2 hours. For older animals an overnight incubation may improve yields. Bone and hair will not be lysed.

3. OPTIONAL: mouse tail snips have low levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. To do so, add 4 µl of RNase A (100 mg/ml) and incubate at room temperature for 2 minutes. Proceed with the following protocol.

4. Centrifuge for 5 minutes at 12,000 x g to pellet insoluble tissue debris and hair. CAREFULLY aspirate the supernatant, and transfer to a sterile 1.5 ml microtube, while leaving behind any insoluble pellet.

5. Add 1 volume Buffer BL followed by 1 volume Absolute Ethanol. Alternatively, user may add 2 volumes of premixed BL/ethanol mixture to the sample. Vortex thoroughly to mix. Thorough mixing is essential at this point.

6. Transfer the entire sample from Step 5 into the column including any wispy precipitate that may have formed.

7. **Centrifuge at 10,000 x g for 1 min to bind DNA. Discard the flow-through liquid.**

8. **Place the Mini Column to the collection tube. Add 500 µl Buffer KB. Centrifuge at 10,000 x g for 1 min. Discard the flow-through liquid.**

9. **Place the Mini Column into the collection tube. Add 500 µl DNA Wash Buffer. Centrifuge at 10,000 x g for 1 min. Discard flow-through liquid**
   Ensure that absolute ethanol has been added to the DNA Wash Buffer before use. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.
   .
10. **Using the same 2 ml collection tube, repeat step 9.**

11. **Using the same 2 ml collection tube, centrifuge the column, with the lid open, at maximum speed of 13,000 x g for 2 min to remove residual ethanol.**
    This step is crucial for ensuring optimal elution in the following step.

12. **Place the Mini Column into a sterile 1.5 ml microtube, and add 100-200 µl of preheated (70°C) Elution Buffer. Allow the sample to sit at room temperature for 3 minutes. Centrifuge at 13,000 x g for 1 min.**

13. ***Optional: Repeat the elution with a second* 100 µl Elution Buffer.**

D. EZgene™ Paraffin-Embedded Tissue Genomic DNA Purification Protocol

1. **Place no more than 30 mg of tissue (~2 mm^3) in a clean 2 ml microtube.**

2. **Extract the sample with 1 ml xylene to remove the paraffin. Mix thoroughly by vortexing.**

3. **Centrifuge the tube at 10,000 x g for 10 min at room temperature. Discard the supernatant without disturbing the pellet.**

4. **Rinse the pellet with 1 ml Absolute Ethanol to remove any traces of xylene. Centrifuge at 10,000 x g for 5 min at room temperature. Discard the ethanol without disturbing the tissue pellet.**

5. **REPEAT the ethanol rinse.**

6. **Air-dry the tissue pellet at 37°C for 15 min.**

7. **Add 200 µl Buffer TL to the tissue and then follow the standard Tissue DNA Isolation Protocol on page 5, starting with step 2 (using reconstituted Proteinase K).**

   For DNA elution, we recommend using 50-100 µl of Elution Buffer warmed to 70°C. Yields will depend on size and age of sample. Certain samples may require prolonged lysis with Buffer TL.

   Tissue fixed with paraformaldehyde will yield degraded DNA or RNA. The extent of degradation depends on type of fixative used, but the size of DNA obtained is usually less than 500 bp. Degradation is not caused by the EZgene™ Tissue DNA Protocol. For PCR detection of segments smaller than 500 bp, satisfactory results can be obtained.

EZgene™ Vacuum/Spin Protocol

Carry out disruption, homogenization, protease digestion, and loading onto the ezBind™ DNA Mini Column as indicated in previous protocols. Instead of continuing with centrifugation, follow the steps outlined below.

NOTE: Please read through previous sections of this book before beginning this protocol.

1. **Prepare the vacuum manifold according to manufacturer's instruction, and connect the ezBind™ DNA V-Spin Column to the manifold.**

2. **Load the sample onto the column.**

3. **Switch on vacuum to draw the sample through the column, and then turn off the vacuum.**

4. **Wash the column by adding 500 µl Buffer KB. Draw the liquid through the column by turning on the vacuum.**

5. **Wash the column by adding 700 µl DNA Wash Buffer. Draw the Wash Buffer through the column by turning on the vacuum source.**

6. **Washing the column with another 700 µl DNA Wash Buffer.**

7. **Place the column into a 2ml collection tube, and transfer the column into a microcentrifuge. Spin for 2 min at 13,000 x g with the lid open to dry the column.**

8. **Place the column into a clean 1.5 ml microtube, and add 100-200 µl of preheated (70°C) Elution Buffer. Let it sit at room temperature for 1-2 min, and then centrifuge at 13,000 x g for 1 min to elute DNA.**

Troubleshooting Guide

Problem	Cause	Possible Solution
Clogged Column	Incomplete Lysis	Add the correct volume of Buffer BL and incubate for specified time at 65°C. It may be necessary to extend incubation time by 10 min.
	Sample is too Large	If using more than 250µl of Blood, increase volumes of Protease, Buffer BL, and Isopropanol. Pass aliquots of lysate through one column successively.
	Sample is too viscous	Divide sample into multiple tubes, and adjust the volume to 250 µl with 10 mM Tris-HCL.
Low DNA Yield 260 /280Low A /A Ratio	Clogged Column	See above
	Poor elution	Repeat elution or increase elution volume (see notes on elution on page 6) Incubation of column at 70°C for 5 min with Elution Buffer may increase yields.
	Improper washing	Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on page 3 before use.
	Buffy Coat Used	With Buffy Coat samples, use absolute ethanol, rather than isopropanol .
	Extended centrifugation during elution.	Resin from the column may be present in eluate and affect the OD absorbance. Avoid centrifugation at speed higher than 15,000 x g. The trace resin in the eluted DNA will not interfere with PCR or restriction digests.
No DNA Eluted	Poor cell lysis due to incomplete mixing with Buffer BL.	Repeat the procedure, this time making sure to vortex the sample with Buffer BL immediately and completely.
	Hemoglobin Remains on column	After application of sample to the column, wash once with 300µl of Buffer BL.
	Poor Cell Lysis due to improper mixing with Buffer BL	Mix thoroughly with Buffer BL prior to loading the column.

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