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Introduction

The EZgene™ Forensic gDNA Purification Kit provides a rapid and easy method for the isolation of genomic DNA from forensic samples, such as dry blood, buccal swabs, and sperm for consistent PCR and Southern analysis. This kit can also be used for the preparation of genomic DNA from mouse tail snips, whole blood, buffy coat, serum, and plasma. No phenol/chloroform extractions and isopropanol or ethanol precipitation are needed. DNA purified using this method is ready for downstream applications such as PCR, Southern blotting, and restriction digestion.

In this procedure, samples are first lysed and applied to the spin column that DNA binds. While cellular debris, hemoglobin, and other proteins are effectively washed away by DNA Wash Buffer, pure DNA is eluted in sterile deionized water or elution buffer. Each ezBind column can bind approximately 100 μg DNA.

Storage and Stability

Once reconstituted, Protease K must be stored at -20℃. All other components can be stored at 22-25℃.Under these conditions, performance of all components of the kit are guaranteed at least 12 months from the date of purchase. Under cool ambient conditions, a precipitate may form in the Buffer BL; heat the bottle at 37℃ to dissolve the precipitate before use.

Safety Information

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

Kit Contents

Product	GD2512-00	GD2512-01	GD2512-02
Preps	4	50	250
DNA Columns	4	50	250
2 mL Collection Tubes	8	100	500
Buffer BL	3 mL	30 mL	150 mL
Buffer TL	3 mL	20 mL	100 mL
Buffer KB	3 mL	28 mL	135 mL
DNA Wash Buffer	2 mL	15 mL	3 x 24 mL
Elution Buffer	2 mL	15 mL	60 mL
Protease K	3 mg	30 mg	5 x 30 mg
User Manual	1	1	1

For isolation gDNA from sperm

Product	GD2512-00	GD2512-01	GD2512-02
10x Buffer A	5 mL	50 mL	250 mL
Buffer B	1 mL	15 mL	70 mL

Note: The kit is supplied with enough buffers for the standard protocol. However, due to increased volumes called for in some protocols (such as the buccal swab protocol), extra buffers can be purchased separately from Biomiga. See product inforamtion on our website or call customer service for price information.

Before Starting

Important:

- Reconstitute Protease K in 110 µL (GD2512-00) or 1.3 mL (GD2512-01) or 5 x 1.3 mL (GD2512-02) Elution Buffer. Vortex the vial and spin the vial briefly prior to use.
- Dilute DNA Wash Buffer with absolute ethanol as follows:
    - **GD2512-00** Add 8 mL ethanol
    - **GD2512-01** Add 60 mL ethanol
    - **GD2512-02** Add 96 mL ethanol/bottle
- Prepare DL-Dithiothreitol (DTT) for Hair, Nails and Feathers protocol.

Protocol for Isolation of DNA from Dried Blood, Body Fluids and Sperm Spots

Dried blood, body fluids, and sperm samples on filter paper can be processed using the following method. Use Specimen paper or blot paper for spotting samples as the filter paper disintegrates DNA when aqueous buffers are added.

1. Punch out the sample spot from the filter paper. (Use less than **200 µL** of blood each spot). Tear or cut filter into small pieces and place into a microfuge tube.

   Note: Use 3 mm diameter cycle puncher for each sample.

2. Add **200 µL Buffer TL** and incubate at 55 ℃ for 15-20 min. Mix well by vortexing 3-5 times during incubation.

3. Add **25 µL Protease K solution** and mix well by votexing. Incubate for at 60 ℃ for 30-60 min with occasional mixing. Spin the sample briefly to collect droplets from the cap.

4. Add **225 µL Buffer BL** and mix well by vortexing. Incubate at 60 ℃ for 10 min. Spin the sample briefly to collect an droplets from the cap.

5. Add **225 µL absolute ethanol** and mix thoroughly by vortexing. Briefly centrifuge to remove any droplets inside the tube.

6. Apply the entire sample into a DNA column, including any precipitate that may have formed. Centrifuge at 10,000 rpm for 1 min. Discard collection tube and flow-through liquid.

7. Transfer the column into a new collection tube and add **500 µL** of **Buffer KB** into column. Centrifuge at 10,000 rpm for 30s. Dispose of flow through liquid and re-use the collection tube.

8. Add **650 µL** of **DNA Wash Buffer**. Centrifuge at 10,000 rpm for 30s. Discard the flow-through liquid and put the column back to the collection tube.

9. Add **650 µL** of **DNA Wash Buffer** and centrifuge as above. Discard flow-through and put the column, **with the lid open**, to a new collection tube.

10. Centrifuge at the column at 13,000 rpm to dry the column. This step is critical for removing residual ethanol that might interfere with DNA yield and purity.

11. Place the column into a sterile1.5 mL tube and add **100 µL** of preheated (70 ℃) **Elution Buffer**. Incubate the tube at 70 ℃ for 3 minutes.

12. Centrifuge at 10,000 rpm for 1 min to elute the DNA.

Note: Add the eluted DNA back to the column for a second elution will yield another 20% of DNA bound.

Note: Incubation at 70 ℃ rather than at room temperature will give a modest increase in DNA yield per elution.

Note: Blood spots from finger pricks usually contain no more than 50 µL blood and yield approximately **0.5 -1 µg** DNA. This is sufficient for PCR analysis.

Protocol for Isolation of Genomic DNA from Sperm

This protocol can be used for fresh or frozen semen samples with equal efficiency.

Frozen samples must to be thawed thoroughly before use. Note that lysis time will vary depending on the size and density of the source material.

Dilute the following buffer before starting:

Buffer A: Dilute 10× Buffer A to 1× Buffer A with ddH_2O before use.

Buffer B: Add ß-mercaptoethanol to final percentage of 2% (v/v).

1. Add **50-250 µL** of **sperm** to **10 mL** of **Buffer A** in a glass (Corex) centrifuge tube. Vortex for 10 seconds at full speed. Only use Corex tubes to prevent attachment of the sperm cells to the tube walls.

2. Centrifuge for 10 min at 4,000 rpm (2500 x g).

3. Carefully remove the supernatant leaving **1 mL** of **pellet** and **Buffer A.**

4. Vortex for 5s and centrifuge at full speed to collect any sample adhering to the walls of tubes. Transfer the sample to a 2.0 mL tube.

5. Add **0.5 mL** of **Buffer A** to the Corex tube and vortex for 30s and centrifuge at full speed to collect any sample adhering to the walls of the tube. Transfer sample to a 2 mL microfuge tube.

6. Centrifuge for 2 min at full speed.

7. Carefully remove the supernatant without disturbing the semen pellet.

8. Resuspend pellet in **200 µL** of **Buffer B**.

9. Add **25 µL Proteinase K** (**25 mg/mL**) and incubate for 2 hours at 60 °.

10. Invert the tube occasionally to disperse the sample or place on a rocking platform.

11. Add **250 µL Buffer BL** and **260 µL ethanol** to the sample and mix by vortexing.

12. Go to step 6 on page 4 and follow the standard protocol to carry out the DNA purification.

Protocol for Isolation of Genomic DNA from Buccal Swabs

Typical yields from cotton or C.E.P. swabs are typically around 0.5 - 3 μg DNA.

1. Follow standard protocol; scrape the swabs firmly against the inside of cheek 5-10 times. Air-dry or vacuum the swabs for 1-2 hours after collection. The person providing the sample should not eat or drink for at least 30 min prior to the sample collection.

2. Remove and transfer the buccal swab into a 2.0 mL tube and add to the tube.

3. Add **525 μL PBS, 25 μL Protease K solution** and **525 μL Buffer BL** to the sample. Mix immediately by votexing for 30s. Incubate 30 min at 60 ℃ with occasional mixing. Briefly centrifuge to remove any droplets from inside the lid.

4. Add **525 μL absolute ethanol** and mix thoroughly by vortexing. Briefly centrifuge to collect any droplets from the lid.

5. Carefully apply **600 μL** of **the mixture** into the column. Centrifuge at 10,000 rpm for 30s. Discard the flow-through liquid and put the column back to the collection tube.

6. Carefully apply the remaining mixture to the column and centrifuge at 10,000 rpm for 30s. Discard the flow-through liquid and collection tube.

7. Put the column into another collection tube, Add **650 μL** of **DNA Wash Buffer** and centrifuge at 10,000 rpm for 1 min. Discard the flow-through liquid and put the column back to the collection tube.

8. Add **650 μL** of **DNA Wash Buffer** and centrifuge as above. Discard the flow-through liquid and collection tube. Put the column, with the lid open, to a new collection tube.

9. Centrifuge at 13,000 rpm for 2 min to dry the column. This step is critical for removal of residual ethanol that might result in decreased DNA yield and purity.

10. Place the column into a nuclease-free 1.5 mL microfuge tube and add **100 µL** of preheated (70 ℃) Elution Buffer. Allow tube to incubate at 70 ℃ for 3 min.

11. Centrifuge at 10,000 rpm for 1 min to elute DNA.

Note: Add the eluted DNA back to the column for a second elution will yield another 20% of DNA bound.

Note: Incubation at 70 ℃ rather than at room temperature will give a modest increase in DNA yield.

Protocol for Isolation of Bacterial gDNA from Biological Fluids

1. Pellet bacteria by centrifuging 10 min at 8,000 rpm.
2. Resuspend the pellet with **200 µL Buffer TL.**
3. Follow the standard protocol (Page 4) from Step 3.

Protocol for Isolation of Genomic DNA from Eye, Nasal, and Other Swabs

1. Collect the sample and add 2 mL PBS. Incubate 2-3 hours at 30 ℃.
2. Pellet bacteria by centrifuging 10 min at 8,000 rpm.
3. Resuspend bacterial pellet with **200 µL Buffer TL.**
4. Follow the standard protocol (Page 4) from Step 3.

Protocol for Isolation of DNA from Salvia

1. Add1.5 mL saliva to a 15 mL centrifuge tube contains 6 mL PBS.
2. Vortex to mix well.
3. Centrifuge at 2000 x g for 5 min. Discard the supernatant and ressuspend the pellet in **180 µL PBS.**
4. Transfer the sample to a new 1.5 mL centrifuge tube.
5. Add **25 µL Protease K solution**, **200 µL Buffer BL.** Add **20 µL RNase A solution** if RNA-free DNA is desired. Mix throughly by vortexing for 10s. Briefly centrifuge to collect any droplets from the lid. Incubate 15 min for 70 ℃ with occasional mixing.
6. Add **200 µL absolute ethanol** and mix throughly by voretxing.
7. Go to Step 6 on page 4 and carry out the purification procedure.

Protocol for Isolation of DNA from Hair, Nails and Feathers

1. Cut the sample into small pieces (0.5-1 cm) and transfer to a 1.5 mL centrifuge tube.

   Note: For hair, cut from base of hair for feathers: select the primary feathers. (Large birds, secondary tail or breast feather can be used).

2. Add **250 µL TL Buffer**, **25 µL Protease K** and **20 µL 1 M DTT**. Mix thoroughly by vortexing. Incubate 30 min at 60 ℃ with occasional mixing.
3. Add **250 µL Buffer BL** to the sample, mix throughly by vortexing. Add **250 µL absolute ethanol** to the sample, mix thoroughly by vortexing.
4. Follow the standard protocol from Step 6 on Page 4.

Vacuum/Spin Protocol

Go through the previous sections of this manual before using this protocol.

1. Prepare samples by following the standard protocol in previous sections (Steps 1-5).

2. Prepare the vacuum manifold according to manufacturer's instructions and connect the column to the manifold.

3. Load the sample/Buffer BL/ethanol mixture into the column.

4. Switch on vacuum source to draw the sample through the column; then turn off the vacuum.

5. Wash the column by adding **650 µL DNA Wash Buffer**. Draw the DNA Wash Buffer through the column by turning on the vacuum source. Repeat this step with another **650 µL DNA Wash Buffer.**

6. Transfer the column, with the lid open, to a 2 mL collection tube. Centrifuge at 13,000 rpm for 1 min to dry the column. It is critical to remove the residual ethanol completely for optimal DNA yield and purity.

7. Place the column in a nuclease-free 1.5 mL microcentrifuge tube and add **50-100 µL Elution Buffer** or **ddH_2O.** Allow standing for 1-2 min, and then centrifuge 1 min to elute DNA.

   Note: Add the eluted DNA back to the column for a second elution will yield another 20% of DNA bound.

   Note: Incubation at 70℃ rather than at room temperature will give a modest increase in DNA yield.

Determination of Yield and Quality

The total DNA yield can be determined by,

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

The quality of DNA can be evaluated by $OD_{260/280}$. A ratio of (A_{260}/A_{280}) of 1.8-1.9 corresponds to 90%-95% purity. Expected yields vary with both amount, and type of tissue used. 30 mg of fresh tissue will yield approximately10-40 µg DNA with two elutions (each 100 µL).

Trouble Shooting Guide

Problem	Possible Cause	Suggestions
Colored residue in column After washing	Forgot to add ethanol	Before applying sample to column, both Buffer BL and ethanol must be added. See protocol above.
	Forgot to add ethanol to DAN Wash Buffer	Dilute DNA Wash Buffer with the indicated volume of absolute ethanol before use.
	Incomplete lysis due to improper mixing with Buffer BL.	Buffer BL is viscous and the sample must be vortexed thoroughly.
	No ethanol added to DNA Wash Buffer Concentrate.	Dilute DNA Wash Buffer with the indicated volume of absolute ethanol before use.
Column clogged	Incomplete lysis	Extend incubation time of lysis with Buffer TL and protease. Add the correct volume of Buffer BL and incubate for specified time at 70℃. It may be necessary to extend incubation time by 10 min.
	Sample too large	If using more than 30 mg tissue, increase volumes of Proteinase K, Buffer TL, Buffer BL, and ethanol. Pass aliquots of lysate through one column successively.
	Sample too viscous	Divide sample into multiple tubes, adjust volume to 250 µL with 10 mM Tris-HCl.
Low DNA yield	Clogged column	See above
	Poor sample release from collection paper	Incubate the OB specimen collection paper longer in TL buffer. Shake the tubes frequently.
	Poor elution	Repeat elution or increase elution volume. Incubation of column at 70℃ for 5 min with Elution Buffer may increase yields.
	Improper washing	DNA Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on Page 3 before use.

Trouble Shooting Guide (Continue from page 13)

Problems	Possible Cause	Suggestions
A_{260}/A_{280} ratio lower than 1.7	Extended centrifugation during elution step.	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation — it will not interfere with PCR or restriction digests.
	Poor cell lysis due to incomplete mixing with Buffer BL	Repeat the procedure, this time making sere to vortex the sample with Buffer BL immediately and completely.
	Incomplete cell lysis or protein degradation due to insufficient incubation.	Increase incubation time with Buffer TL and protease. Ensure that no visible pieces of tissue remain.
	Samples are rich in protein.	After applying to column, wash with 300 µL of a 1:1 mixture of Buffer BL and ethanol and then with DNA Wash Buffer.
No DNA eluted	Poor cell lysis due to improper mixing with Buffer BL.	Mix thoroughly with Buffer BL prior to loading ezBind column.

Related EZgene™ Products

Catalog #	Product Name	Preps	Price $
GD2211-01	Tissue gDNA kit	50	90.00
GD2211-02	Tissue gDNA kit	250	420.00
GD2814-01	96-well tissue gDNA kit	4x96	680.00
GD2814-02	96-well tissue gDNA kit	20x96	3000.00
GD2311-01	Blood gDNA mini kit	50	90.00
GD2311-02	Blood gDNA mini kit	250	420.00
GD2312-01	Blood gDNA midi kit	10	90.00
GD2312-02	Blood gDNA midi kit	25	200.00
GD2314-01	Blood gDNA maxi kit	10	160.00
GD2314-02	Blood gDNA maxi kit	25	360.00
GD2815-01	96-well blood gDNA kit	4x96	680.00
GD2815-02	96-well blood gDNA kit	20x96	3000.00
GD2411-01	Bacterial gDNA kit	50	110.00
GD2411-02	Bacterial gDNA kit	250	495.00
GD2412-01	Soil gDNA kit	50	90.00
GD2412-02	Soil gDNA kit	250	420.00
GD2413-01	Insect gDNA kit	50	90.00
GD2413-02	Insect gDNA kit	250	420.00
GD2414-01	Mullusc gDNA kit	50	90.00
GD2414-02	Mullusc gDNA kit	250	420.00
GD2415-01	Yeast gDNA kit	50	90.00
GD2415-02	Yeast gDNA kit	250	420.00
GD2416-01	Fungal gDNA kit	50	90.00
GD2416-02	Fungal gDNA kit	250	420.00
GD2512-01	Forensic gDNA purification kit	50	85.00
GD2512-02	Forensic gDNA purification kit	250	380.00

Limited Use and Warranty

This product is intended for in vitro research use only. Not for use in human.
This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technology support or learn more product information, please visit our website at www.biomiga.com or contact us at (858) 603-3219.

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