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XcelGen | Blood gDNA Mini Kit

User Guide

Cat No: XG2311-01

XcelGen

Ver.: 0.2/13

Revised Protocol

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Introduction

The Blood gDNA kit provides a fast and easy method for isolating gDNA from blood. The system utilizes the reversible nucleic acid-binding properties of membrane and the speed of spin column technology to yield high quality gDNA with the OD_{260}/OD_{280} ratio of 1.8-2.0. This Kit can also be used for the preparation of genomic DNA from buffy coat, serum, plasma, saliva, buccal swab and other body fluids. Purified DNA is suitable for applications such as PCR, Southern Blotting and Restriction Digestion. The binding capacity per column is 40 µg of gDNA.

Overview

If using the Blood gDNA Mini Kit for the first time, please read this booklet to become familiar with the procedures. Samples are homogenized and lysed in a high salt buffer. The DNA is bound to the column while proteins and other impurities are removed by wash buffer. The purified DNA is suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization techniques.

Storage and Stability

All components of the Blood gDNA Mini Kit are stable for at least 12 months when stored at 22°C-25°C. Protease K should be stored at -20°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer SBL and Buffer BL. It is possible to dissolve such deposits by warming the solution at 55°C, though we have found that they do not interfere with overall performance.

Kit Contents

Product	XG2311-00	XG2311-01
Preps	4	50
DNA Mini Columns	4	50
2 ml Collection Tubes	8	100
Buffer SBL	1.5 ml	20 ml
Buffer MB	1 ml	12 ml
Buffer BL	1.8 ml	22 ml
Red Blood Cell Lysis Buffer	12.5 ml	125 ml
DNA Wash Buffer	2 ml	15 ml
Elution Buffer	1 ml	15 ml
Protease K	90 µl	1.2 ml
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Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important

- Dilute Buffer MB: Add 1.5 ml (XG2311-00) or 18 ml (XG2311-01) of absolute ethanol to each bottle.
- Dilute DNA Wash Buffer: Add 8 ml (XG2311-00) or 60 ml (XG2311-01) of absolute ethanol to each bottle.
- Under cool ambient conditions, precipitates may form in Buffer SBL. In case of such an event, heat the bottle at 55°C to dissolve before use.
- All centrifugation steps must be carried out at room temperature.
- Buffer SBL contains 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 eyewear when handling.

Blood gDNA Mini Kit Protocol

A. Blood & Body Fluid DNA Protocol

Note: *This protocol is optimized for isolating gDNA from 200~400 µl fresh or frozen blood samples. 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 processed using this procedure.* The expected yield from 200 µl of blood is approximately **4-12 µg** of DNA.

1. Transfer the Blood sample to the tube.

   Note: *Add 1-2.5 volume Red Blood Cell Lysis Buffer (provided), invert 5 times to mix well. Centrifuge at 10,000 rpm for 1 minute. Aspirate supernatant, and add 200µl with PBS or Elution Buffer (provided) to the cell pellet. Repeat lysis if it is necessary. Vortex to mix completely.*

   Note: *For isolating genomic DNA from Avian, Bird, and Amphibian, decrease the sample volume to 5-25µl, and bring the volume up to 200µl with PBS or Elution Buffer (provided).*

2. Add **20µl of proteinase K** to a 1.5 ml microfuge tube.
3. Add **1 Volume of Buffer SBL** (200µl of Buffer SBL to 200µl of Blood Sample). Mix well by vortexing at maximum speed for 20s.

   Note: *Precipitates may form in Buffer SBL. Dissolve at 55°C before use.*

4. Incubate the sample at 55°C for 10 minutes. Briefly vortex the tube once during incubation.
5. Add **1 Volume of absolute ethanol** (200µl of absolute ethanol to 200µl of Blood Sample) to the lysate. Vortex at maximum speed for 10s. Briefly centrifuge the tube to collect any drops from the lid.

   Note: *Add mix solution of 1:1 Volume of absolute ethanol to isopropanol may achieve better than absolute ethanol. For instance, mix solution of 100µl absolute ethanol and 100µl isopropanol may achieve better than 200µl of absolute ethanol.*

6. Add **400µl of Buffer BL** into the spin column (Provided), incubate at room temperature for 2 min, centrifuge at 12,000 rpm for 2 min, and discard the flow through. The column is ready and work well for binding DNA.

7. Transfer the sample to the DNA column, and centrifuge at 12,000 rpm for 1 min. Discard the collection tube and flow-through liquid.
8. Place the column into a new collection tube. Add **450µl of Buffer MB**(Add ethanol before use), and centrifuge at 12,000 rpm for 30s. Discard the collection tube and flow through liquid and put the column back to the collection tube.
9. Add **650µl of DNA Wash Buffer** (Add ethanol before use). Centrifuge at 12,000 rpm for 30s. Discard the collection tube and flow-through liquid.

   Note: *DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle label, and on page 2. Using a new 2 ml collection tube, repeat step 9 with another* ***650µl DNA Wash Buffer*** *and centrifuge as above. Discard the flow-through.*
10. Put the empty column, with the lid open, into the same 2ml collection tube and centrifuge at 12,000 rpm for 5 min to dry the column.

    Note: *Residual ethanol will be removed more efficiently with the lid open. It is critical to remove the ethanol before elution.*
11. Place the column into a sterile 1.5ml microfuge tube, add **50-100µl** preheated (65°C) **Elution Buffer**. Incubate at room temperature for 2 min.
12. Centrifuge at 13,000 rpm for 1 min to elute the DNA. The first elution normally yields 60-70% of DNA bound. A second elution with another **50-100µl Elution Buffer** will yield another 20% of the DNA.

B. Blood & Body Fluid DNA Vacuum/Spin Protocol

1. Prepare the lysate and column as described step 1-6 on page 4.
2. Insert a DNA column into the vacuum manifold. Carefully apply the lysate to the column. Turn on the vacuum source to draw all of the liquid through the column.

   Note: *If the lysate has difficulty passing through the column, place the column into a collection tube and centrifuge at 13,000 rpm for 2 min Place the column into another collection tube (supplied), and proceed to step 7 of the spin protocol on page 5.*
3. Add **450µl Buffer MB** to the column and allow the liquid pass through the column by vacuum.
4. Wash the column with **650µl of DNA Wash Buffer**. Repeat once and turn off the column.
5. Transfer the column, with the lid open, to the collection tube and centrifuge at 13,000 rpm for 2 minutes to completely dry the membrane.
6. Transfer the column into a sterile 1.5ml microfuge tube and add **50-100µl** of preheated (65°C) **Elution Buffer**. Incubate the tube at 65°C for 2 min.
7. Centrifuge the tube at 13,000 rpm for 1 min to elute the DNA. Elute DNA again as indicated in the previous step.

   Note: *The first elution typically yields 60-70% of the DNA bound. A second elution with another 50-100µl Elution Buffer will yield another 20% of the DNA. Thus two elution will generally give > 90% yields. To obtain DNA at higher concentrations, the elution can be carried out using 50µl - 100µl of Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50µl greatly reduce yields.*

Troubleshooting Guide

Problems	Possible reason	Suggestions
Clogged Column	Incomplete Lysis	Add the correct volume of Buffer SBL and incubate for specified time at 55°C. It may be necessary to extend incubation time by 10 min.
	Sample is too Large	If using more than 400µl of Blood, increase volumes of Proteinase K, Buffer SBL, and Isopropanol. Pass aliquots of lysate through one column successively.
	Sample is too viscous	Divide sample into multiple tubes, and adjust the volume to 200µl with 10 mM Tris-HCl.
Low DNA Yield	Clogged Column	See above
	Poor elution	Repeat elution or increase elution volume (see notes on elution on page 5) 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 .
Low A260/280 Ratio	Extended centrifugation during elution.	Resin from the column may be present in elute and affect the OD absorbance. Avoid centrifugation at speed higher than 15,000g. The trace resin in the eluted DNA will not interfere with PCR or restriction digests.
	Poor cell lysis due to incomplete mixing with Buffer SBL.	Repeat the procedure, this time making sure to vortex the sample with Buffer SBL immediately and completely.
	Hemoglobin Remains on column	After application of sample to the column, wash once with 300µl of Buffer SBL.

Troubleshooting Guide

Problems	Possible reason	Suggestions
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer SBL	Mix thoroughly with Buffer SBL prior to loading the column.
	Absolute ethanol not added to Buffer SBL.	Before applying the sample to the column and aliquot of Buffer SBL/ethanol solution must be added.
	No Ethanol added to Wash Buffer Concentrate	Dilute Wash Buffer with the indicated volume of absolute ethanol before use (page 3).
Washing Leaves Colored Residue in Column	Incomplete Lysis due to improper mixing with Buffer SBL.	Buffer SBL is viscous and the sample must be mixed thoroughly.
	No Ethanol added to Wash Buffer Concentrate	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.
Eluted Material has Red or Brown Color	Sample Volume is too Large	Reduce sample volume, and proceed with protocol.
	Hemoglobin remains in column	After applying sample, wash column once with 300µl of Buffer SBL.

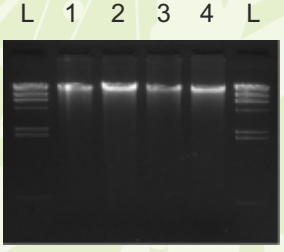

Fig: Agarose gel analysis of Blood gDNA purified with XcelGen Blood gDNA mini Kit.

Lane 1: gDNA isolated from fresh blood sample.
Lane 2: gDNA isolated from frozen blood sample.
Lane 3: gDNA isolated from dried blood sample.
Lane 4: gDNA isolated from clotted blood sample.

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 XcelGen'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 XcelGen. XcelGen's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of XcelGen, to replace the products, XcelGen 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 technical support or for more product information, visit our website at

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