EasyPrep™ Genomic DNA Miniprep Manual

- Animal tissues, cells
- Whole blood, serum and plasma

Catalog#: GD01-01, GD01-02
GD02-01, GD02-02

Bioland

For research use only

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Introduction

The EasyPrep™ Genomic DNA Kit provides a simple and rapid method for the isolation of genomic DNA from a wide variety of biological materials, including cultured cells, tissues, whole blood, plasma, serum, and body fluids.

Instead of using proteinase K to digest the biological materials, the kit applies the lysis buffer directly to the cells or tissues. After homogenization, the lysates are transferred to the binding columns. Thus the time-consuming steps of material preparation are eliminated. The DNA-binding-specific matrix in the column allows the high efficient binding of DNA, while RNA, proteins and other cellular components are easily removed during washing. Purified DNA can be directly used for most applications such as PCR, Southern blotting, sequencing and restriction enzyme digestion.

Kit content

Catalog No.	GD01-01	GD01-02	Storage
Preps	50	250	-
DNA columns	50	250	RT
GD Lysis Buffer	60 ml	260 ml	RT
DNA Wash Buffer	12 ml	50 ml	RT
DNA Elution Buffer	10 ml	25 ml	RT
Column Buffer	10 ml	30 ml	RT
Collection tubes	50	250	RT
Manual	1	1	

CAUTION! Lysis Buffer contains Chaotropic salts. Wear gloves when handling this solution.

Storage and Stability

The EasyPrep™ Genomic DNA kit components are guaranteed for at least 12 months from the date of purchase .

Specifications:

- Tissue and cells: up to 30 mg of tissues or 1-5x10^6 cells can be processed by using the kit
- DNA sizes: up to 40 kb of DNA can be purified from this kit . The purity of the DNA can be measured with expecting A_{260}/A_{280}≥1.8
- DNA yield depends on the biological materials used. Up to 30 μg of genomic DNA can be eluted

Buffer preparation

It is strongly advised that you familiarize yourself with the entire booklet before starting.

1. Add β-mercaptoethanol to the Lysis Buffer to 1% (v/v) before use, then store the buffer at 4°C.
2. Add 96-100% ethanol to DNA wash buffer bottle as follow:

   GD01-01: Add 48 ml absolute ethanol (96-100%) to each bottle

   GD01-02: Add 200 ml absolute ethanol (96-100%) to each bottle
3. Preheat the Elution Buffer to 70°C will increase the yield.

Materials supplied by users:

- Homogenizer
- 96-100% ethanol
- 1.5 ml microcentrifuge tubes
- High speed microcentrifuge

Limited use and warranty

This product is warranted to perform as described in its labeling and in Bioland'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 Bioland. Bioland's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Bioland, to replace the products, Bioland 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.

Protocol for Tissues

1. **Tissue preparation and lysis:**
   - **Using Homogenizer:** Using a blade to slice tissue, weigh out 10-30 mg of animal tissue and transfer it to a microcentrifuge tube, **add 500 µl Lysis Buffer (with β-mercaptoethanol added), homogenize the tissue according to the manufacturer's instruction.**
   - **Using liquid N2:** immediately place the weighted tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Pour tissue powder and liquid nitrogen into a microcentrifuge tube. Allow the liquid nitrogen to evaporate, but don't let the tissue to thaw. **Add 500 µl Lysis Buffer, homogenize through a 20-gauge needle fitted to a syringe.**
   - **Proteinase K digested tissue lysate (up to 125 µl), add 500 µl Lysis Buffer and mix well**.
2. **Centrifuge the homogenized tissue lysate at 11,000 rpm for 5 min.** ***(Optional):*** **During centrifugation, add 100 µl Column Buffer to the column, incubate for 2 min, centrifuge at 11,000 rpm for 1 min.** *This ensures the column having optimal binding capacity. Especially when the kit is stored for a while.*
3. **Transfer the supernatant to a gDNA binding column.** *Don't disturb the pellet during transfer.* **Centrifuge at 11,000 rpm for 1 min. Discard the flowthrough.**
4. **Add 400 µl Lysis Buffer to the column, centrifuge at 11,000 rpm for 1 min. Discard the flowthrough.**
5. **Add 700 µl DNA Wash Buffer to the column, centrifuge at 11,000 rpm for 1 min. Discard the flowthrough.** *Make sure that ethanol is added to washing buffer as instructed before use.*
6. **Centrifuge additional 1 min at 11,000 rpm.** *It is critical to remove residual ethanol for optimal elution. Residual ethanol may interfere subsequent applications.*
7. **Add 100-200 µl Elution Buffer to the center of the column, incubate for 1 min, centrifuge at 11,000 rpm for 1 min to elute the DNA.** *Pre-heat (70°C waterbath) elution buffer will increase the DNA yield.*
8. **Recommended: For maximum DNA yield, repeat elution once as described in Step 7.**

Protocol for Cells

1. **Cell preparation and lysis:**
   - Adherent cells: scrape 1-5x10^6 cultured cells from the culture dish or plate, pellet cells by centrifugation. Completely remove the supernatant by aspiration.
   - Suspension cells: pellet 1-5x10^6 cells by centrifugation. Completely remove the supernatant by aspiration.
   - **Add 500 µl Lysis Buffer, vortex to resuspend the cell pellet. Homogenize the lysate by passing it through a 20-gauge needle fitted to a DNase free syringe for at least 5 times.**
2. **Column equilibration: add 100 µl Column Buffer to the gDNA binding column, incubate for 2 min, centrifuge at 11,000 rpm for 1 min.** *This ensures the column having optimal binding capacity. Especially when the kit is stored for a while.*
3. **Transfer the homogenized lysate to a gDNA binding column. Centrifuge at 11,000 rpm for 1 min. Discard the flowthrough.**
4. **Add 400 µl Lysis Buffer to the column, centrifuge at 11,000 rpm for 1 min. Discard the flowthrough.**
5. **Add 700 µl DNA Wash Buffer to the column, centrifuge at 11,000 rpm for 1 min. Discard the flowthrough.** *Make sure that ethanol is added to washing buffer as instructed before use.*
6. **Centrifuge additional 1 min at 11,000 rpm.** *It is critical to remove residual ethanol for optimal elution. Residual ethanol may interfere subsequent applications.*
7. **Place the DNA column in a clean 1.5 ml microcentrifuge tube. Add 100-200 µl Elution Buffer to the center of the column, incubate for 1 min, centrifuge at 11,000 rpm for 1 min to elute the DNA.** *Pre-heat (70°C waterbath) elution buffer will increase the DNA yield.*
8. **Recommended: For maximum DNA yield, repeat elution once as described in Step 7.**

Protocol for Whole blood, Serum and Plasma

The protocol is used to purify genomic DNA from 100-200 µl whole blood, serum or plasma. Whole blood in fresh, frozen or preserved (such as in EDTA, heparin) forms can be used. If the samples can't be processed immediately, the samples can be preserved at 4°C or –20°C after addition of lysis buffer.

1. **Add 500 µl Lysis Buffer** to the 100 µl whole blood, serum or plasma, mix completely by **vortexing 5 sec, incubate for 5 min.**

2. **Column equilibration: add 100 µl Column Buffer to the gDNA binding column, incubate for 2 min, centrifuge at 11,000 rpm for 1 min.** *This ensures the column having optimal binding capacity. Especially when the kit is stored for a while.*

3. **Transfer the lysate to a gDNA binding column. Centrifuge at 11,000 rpm for 1 min. Discard the flowthrough.**

4. **Add 400 µl Lysis Buffer to the column, centrifuge at 11,000 rpm for 1 min. Discard the flowthrough.**

5. **Add 700 µl DNA Wash Buffer to the column, centrifuge at 11,000 rpm for 1 min. Discard the flowthrough.** *Make sure that ethanol is added to washing buffer as instructed before use.*

6. **Centrifuge additional 1 min at 11,000 rpm.** *It is critical to remove residual ethanol for optimal elution. Residual ethanol may interfere subsequent applications.*

7. **Place the DNA column in a clean 1.5 ml microcentrifuge tube. Add 100-200 µl Elution Buffer to the center of the column, incubate for 1 min, centrifuge at 11,000 rpm for 1 min to elute the DNA.** *Pre-heat (70°C waterbath) elution buffer will increase the DNA yield.*

8. **Recommended: For maximum DNA yield, repeat elution once as described in Step 7.**

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