Bio-Rad
Laboratories

mD_x® Product Line

InstaGene™ Genomic DNA Kit

Instruction Manual

Catalog Number 732-6028
100 DNA preparations starting from 300 µl of whole blood

Please familiarize yourself with the contents of this insert before using the product for the first time.

1. INTRODUCTION

The InstaGene Genomic DNA kit is used to purify double-stranded genomic DNA from whole blood. The kit may be used with blood collected in EDTA, heparin, or citrate. The purified DNA can be used for Southern blotting or as template for amplification reactions. The procedure is written for the isolation of DNA from 300 µl of whole blood. However, any volume of blood can be used by simply increasing or decreasing the volume of the kit components proportionately. For optimum results, use blood that has been refrigerated at 2-8 °C for no more than 1 week, or frozen at -20 °C for no more than 2 weeks. Typical DNA yield from 300 µl of whole blood is 5-10 µg. Average length of DNA is > 50 kb.

2. KIT COMPONENTS AND STORAGE

Component	Description	Amount	Storage Temperature
Lysis Solution 1	Solution of inorganic salts. Irritant.	230 ml	15-30 °C
Lysis Solution 2*	Solution of anionic detergent. Irritant.	2 ml	15-30 °C
Protein Precipitation Solution	Solution of organic salt. Irritant.	25 ml	15-30 °C
DNA Rehydration Solution	Solution of inorganic salts. Irritant.	25 ml	15-30 °C

* Note: Lysis Solution 2 may form a precipitate during storage. Warm the bottle at 37-55 °C for a few minutes to dissolve the precipitate.

3. ITEMS REQUIRED BUT NOT PROVIDED

Available from Bio-Rad

- 1.5 ml microcentrifuge tubes (Cat. No. 223-9501).
- Micropipette tips, 20-200 µl and 100-1000 µl (Cat. No. 211-2016 and 211-2021, respectively).

Not available from Bio-Rad

- Adjustable micropipettes, 20-200 µl and 100-1000 µl
- 95% or absolute ethanol
- 70% ethanol
- Microcentrifuge
- Vortex mixer
- Disposable gloves

4. SAFETY

4.1 The Centers for Disease Control recommend the use of "universal precautions" when handling blood and certain body fluids. Under universal precautions, blood and certain body fluids of all patients are considered potentially infectious for Hepatitis B Surface Antigen (HbsbAg), HIV-1/2, HTLV-1/2, HCV, HBV and other bloodborne pathogens. It is recommended that workers wear protective barriers such as gloves, gowns, aprons, masks or protective eyewear to reduce the risk of exposure of the worker's skin or mucous membranes to potentially infective materials.

4.2 Kit reagents contain irritants, including: inorganic salts, organic salt and anionic detergent (See Product Safety Information, page 5). Please refer to the Material Safety Data Sheets for instructions on proper handling and disposal of these reagents.

5. SUMMARY OF THE PROCEDURE

Lysis 1	Add 900 µl of Lysis Solution 1 to a 1.5 ml tube. Add 300 µl of whole blood. Mix by inverting tube 10-15 times. Centrifuge at 13,000-15,000 x g for 20 seconds. Remove and discard supernatant.
Wash 1	Resuspend pellet by pipetting up and down 500 µl of Lysis Solution 1. Centrifuge at 13,000-15,000 x g for 20 seconds. Remove and discard supernatant. Repeat for a total of 2 times..
Lysis 2	Resuspend pellet by pipetting up and down 200 µl of Lysis Solution 1. Add 15 µl of Lysis Solution 2. Mix by pipetting up and down.
Precipitation 1	Add 200 µl of Protein Precipitation Solution. Vortex 10-20 seconds. Centrifuge at 13,000-15,000 x g for 3 minutes.
Precipitaion 2	Transfer supernatant to a 1.5 ml tube. Add 400 µl of 95 % ethanol. Mix by inverting tube 10-20 times. Centrifuge at 13,000-15,000 x g for 20 seconds.
Wash 2	Add 500 µl of 70% ethanol and invert tube 5-10 times. Centrifuge at 13,000-15,000 x g for 20 seconds. Remove and discard supernatant.
Drying	Air dry pellet for 5 -10 minutes.
Rehydration	Add 20-100 µl of Rehydration Solution.

6. EXTRACTION PROCEDURE

6.1 Add 900 µl of Lysis Solution 1 to a sterile 1.5 ml microcentrifuge tube.

6.2 Add 300 µl of whole blood to the 1.5 ml tube containing Lysis Solution 1 and mix by inverting the tube 10-15 times.

6.3 Centrifuge at 13,000-15,000 x g for 20 seconds. **Remove and discard as much of the supernatant as possible without disturbing the white cell pellet.**

6.4 Wash the white cell pellet **twice** by adding 500 µl of Lysis Solution 1 and pipetting up and down until the pellet is resuspended. Centrifuge at 13,000-15,000 x g for 20 seconds. **Remove and discard as much of the supernatant as possible without disturbing the white cell pellet.**

6.5 Resuspent the white cell pellet in 200 µl of Lysis Solution 1 by pipetting up and down. **It is very important that the pellet be completely resuspended before proceeding to the next step.**

6.6 Add 15 µl of Lysis Solution 2 and mix by pipetting up and down and finger vortexing.

6.7 Add 200 µl of Protein Precipitation Solution and vortex for 10-20 seconds. Centrifuge at 13,000-15,000 x g for 3 minutes to precipitate the proteins.

6.8 Transfer the supernatant to a sterile 1.5 ml microcentrifuge tube and add 400 µl of 95% ethanol to precipitate the DNA. Mix by inverting the tube 10-20 times or until white thread-like strands of DNA are visible.

6.9 Centrifuge at 13,000-15,000 x g for 20 seconds. The DNA will be visible as a small white pellet. Carefully remove the supernatant.

6.10 Add 500 µl of 70% ethanol and invert the tube 5-10 times to wash the DNA pellet. Centrifuge at 13,000-15,000 x g for 20 seconds. Carefully remove the supernatant. If needed, centrifuge the tube again and remove any remaining liquid.

6.11 Invert the tube on clean absorbent paper to remove traces of liqid on the walls of the tube. **Air dry the pellet for 5-10 minutes.**

6.12 Add 20-100 µl of DNA Rehydration Solution. Complete rehydration of the DNA typically requires overnight incubation at room temperature.

6.13 Store the DNA at 2-8 °C.

7. AMPLIFICATION REACTION PROCEDURE

7.1 A typical amplification reaction will consist of 50-250 ng of the extracted DNA template in a reaction volume of 25-50 µl.

7.2 Amplification reaction conditions have to be optimized for specific applications.

8. TROUBLESHOOTING GUIDE

DNA prepared using the InstaGene Genomic DNA Kit is PCR-ready, is restrictable (Southern blots and enzyme digests), and is clonable. However, there is one main cause for low amplification yield.

8.1 Incomplete removal of ethanol after the last wash step. To obtain optimal yield, it is necessary that all traces of ethanol be removed from the microcentrifuge tube. Air dry the pellet completely as described in Step 6.11.

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