mD$_x$™ Product Line

InstaGene™ Whole Blood Kit

Instruction Manual

Catalog Number
732-6211

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4100074 Rev B

BIO-RAD

Introduction

The InstaGene whole blood DNA isolation kit is used to purify genomic DNA from whole blood for subsequent use as template in amplification reactions. The yield is 1–2 µg of template DNA from 50 µl of whole blood.

Kit Contents

(enough for 100 DNA preps)

Lysis Buffer, 200 ml
InstaGene Matrix, 20 ml

Storage Conditions

The kit should be stored at 2–8 °C.

Items Required But Not Provided

70 °C heat block (or 70 °C water bath)

95 °C heat block (or boiling water bath)

1.5 ml microcentrifuge tubes, preferably screw-capped, catalog number 224-0110

Disposable fine-tip transfer pipets, catalog number 223-9528

Microcentrifuge, preferably with fixed-angle rotor

Vortex mixer

Magnetic stir plate

Summary of the Procedure-PCR*-Ready DNA

RBC Lysis	Mix 50 µl whole blood + 1 ml Lysis Buffer Incubate: 8 min at RT Spin: 1 min at RT Aspirate off supernatant
Wash 1	Add 0.5 ml Lysis Buffer Vortex Spin: 1 min at RT Aspirate off supernatant
Wash 2	Add 0.5 ml Lysis Buffer Vortex Spin 1 min at RT Aspirate off supernatant
InstaGene Step	Add 0.2 ml InstaGene matrix Incubate 8 min at 70 °C Vortex Incubate: 4 min at 95 °C Vortex Spin 1 min at RT

Procedure–PCR-Ready DNA

Note: Because of the high-temperature incubation step in the extraction procedure, screw-capped microcentrifuge tubes, catalog number 224-0110 are recommended.

1. Set one heat block at 70 °C and another at 95 °C. (Water baths may be used instead of heat blocks.)
2. In a 1.5 ml microcentrifuge tube, mix 50 µl of blood with 1 ml of lysis buffer. Cap the tube and invert to mix. Let stand at room temperature for 8 minutes with occasional mixing.
3. Centrifuge the tube at 10,000–12,000 rpm for 1 minute. If using a fixed-angle rotor, mark one side of the tube before the centrifugation to easily locate the resulting pellet. Load the tube into the rotor such that the the marked side is away from the center of the rotor. The pellet will then be deposited on this side.
4. Inspect the bottom of the tube for a white to pinkish pellet. To remove the supernatant using a disposable fine-tip transfer pipet, slide the pipet tip down the wall of the tube opposite the side containing the pellet. Remove all but 5–10 µl of the supernatant without disturbing the pellet. Discard the pipet and supernatant.
5. Add 0.5 ml of lysis buffer to the pellet. Vortex at high speed for 10 seconds. Centrifuge at 10,000–12,000 rpm for 1 minute. The pellet should be more visible at this step. Aspirate off as much of the supernatant as possible without touching the pellet.
6. Repeat Step 5. At the end of this step the pellet should be white.

7. The bottle of InstaGene matrix comes with a magnetic stir bar. Place the bottle on a magnetic stir plate and stir at moderate speed for 30 seconds. A uniform suspension should result. Using a large-bore pipet tip (*e.g.* a 1,000 µl tip), immediately remove 200 µl of InstaGene matrix from the bottle and add to the tube containing the pellet. Cap the tube tightly and incubate in the 70 °C heat block for 8 minutes.

8. Vortex the tube at high speed for 10 seconds. Transfer the tube to the 95 °C heat block and incubate for 4 minutes.

9. Vortex the tube at high speed for 10 seconds. Centrifuge at 10,000-12,000 rpm for 1 minute. Use the supernatant for the PCR (typically, 5–10 µl of supernatant in a 50 µl reaction). Be careful not to pipet InstaGene matrix when transferring DNA-containing supernatant to the amplification reaction tube. If the amplification step will not be performed immediately, proceed to Step 10.

10. To save unused portions of DNA, store the tube containing the extracted DNA and InstaGene matrix at -20 °C. Prior to setting up the amplification reaction, thaw the contents of the tube at room temperature, then perform Step 9.

Procedure–LCR**-Ready DNA

The procedure is the same as for PCR-ready DNA, except for the following modifications:

Step 2. Start with 250 µl of whole blood.

Step 3. Centrifuge for 3 minutes.

Step 7. Add 50 µl of InstaGene matrix to the nuclear pellet.

Step 9. Use 5 µl of the supernatant in a 25 µl LCR.

Troubleshooting

DNA prepared using the InstaGene whole blood kit is PCR- and LCR-ready. However, there are two common errors that can cause low DNA extraction efficiency or low amplification yield:

1. Loss of the pellet during the lysis and wash steps. To prevent this, determine the location of the pellet before removing the supernatant. Use a fine-tip pipet and do not touch the pellet. If the pellet is difficult to see after the initial centrifugation, leave 5–10 µl of supernatant at this step (Step 4).
2. Transfer of InstaGene matrix to the amplification reaction tube. This can be prevented by centrifuging the tube containing the DNA and InstaGene matrix just before removing an aliquot of the supernatant (Step 9). Do not disturb the bed of InstaGene matrix at the bottom of the tube.

Product Information

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The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffmann-LaRoche. Use of the PCR process requires a license.

** Ligase Chain Reaction