Bio-Rad Laboratories

mD_x® Product Line

InstaGene™ Dry Blood Kit

Instruction Manual

Catalog Number 732-6212
125 DNA preparations from 1/8"-diameter dry blood spots

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

1. INTRODUCTION

The InstaGene Dry Blood Kit is used to purify genomic DNA from blood that has been spotted onto filter paper (e.g., Schleicher and Schuell #903™ filter paper). The 45 minute procedure results in the selective extraction of amplification reaction inhibitors present in blood without removing any of the filter-bound DNA. The extracted dry blood spot (DBS) is added directly to the amplification reaction mixture prior to thermocycling.

2. KIT COMPONENTS AND STORAGE

Component	Description	Amount	Storage Temperature
Extraction Reagent	Solution of ethanol and chaotrope. Flammable.	25 ml	15-30 °C
Wash Reagent	Solution of ethanol and salts. Flammable.	50 ml	15-30 °C

3. ITEMS REQUIRED BUT NOT PROVIDED

Available from Bio-Rad

- 1.5 ml microcentrifuge tubes (Cat. No. 223-9501).
- Disposable fine-tip transfer pipettes (Cat. No. 223-9528).
- Micropipette tips, 20-200 µl and 100-1000 µl (Cat. No. 211-2016 and 211-2021, respectively).

Not available from Bio-Rad

- Paper punch, 1/8" or 1/4" punch diameter
- Adjustable micropipettes, 20-200 µl and 100-1000 µl
- Sterile distilled or deionized water
- 95% or absolute ethanol
- 65°C heat block (or 65°C water bath)
- Vortex mixer
- Disposable gloves
- SpeedVac™ concentrator (recommended)

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 potentially harmful substances, including ethanol and guanidinium thiocyanate (See Product Safety Information, page 4). Please refer to the Material Safety Data Sheets for instructions on proper handling and disposal of these reagents.

5. SUMMARY OF THE PROCEDURE

Wash 1	Place DBS in a 1.5 ml tube. Add 1 ml water. Incubate 10 minutes at 15-30°C. Shake occasionally. Remove and discard supernatant. Repeat for a total of 2 times.
Extraction	Add 100 µl Extraction Reagent. Incubate 10 minutes at 65°C. Mix occasionally. Remove and discard supernatant. Repeat for a total of 2 times..
Wash 2	Add 100 µl Wash Reagent. Vortex briefly. Remove and discard supernatant. Repeat for a total of 3 times.
Wash 3	Add 100 µl ethanol. Vortex briefly. Remove and discard the supernatant.
Drying	Evaporate off ethanol (SpeedVac concentrator, 10 minutes; or air dry, at least 30 minutes).

6. EXTRACTION PROCEDURE

6.1 Place the DBS sample (one 1/8"-punch) in a 1.5 ml microcentrifuge tube. Add 1 ml of water. Incubate at 15-30°C for 10 minutes with occasional inversion of the tube. Pipet off the supernatant and discard.

6.2 Repeat Step 6.1.

6.3 Add 100 µl Extraction Reagent. Incubate at 65°C for 10 minutes with occasional mixing. **Pipet off the supernatant, including any liquid that might have condensed inside the tube cap, and discard.**

6.4 Repeat Step 6.3. The filter should appear slightly brown or white after the second extraction step.

6.5 Add 100 µl Wash Reagent. **To ensure that all the inside surfaces of the tube are washed, vortex for 1 second while the tube is in the upright position, then vortex again for 1 second while the tube is in the inverted position (make sure the tube is tightly capped!). Pipet off the supernatant, including any liquid inside the cap, and discard.**

6.6 Perform Step 6.5 two more times.

6.7 Add 100 µl ethanol. Vortex as in Step 6.5. Pipet off the supernatant, including any liquid inside the cap, and discard.

6.8 Place the tube containing the filter in a SpeedVac concentrator and dry the filter under vacuum for 10 minutes. Alternatively, air dry the filter for 30 minutes or longer.

7. AMPLIFICATION REACTION PROCEDURE

7.1 A typical amplification reaction will consist of one-half of an extracted 1/8" punch in a 25 µl reaction volume, or the whole 1/8" punch in a 50 µl reaction volume.

7.2 Amplification reaction conditions have to be optimized for specific applications. Conditions found suitable for DNA templates in solution usually apply without further modification when using filter-bound DNA template prepared with this kit.

7.3 Make sure that the filter paper is completely immersed in the reaction mixture.

8. TROUBLESHOOTING GUIDE

DNA prepared using the InstaGene Dry Blood Kit is PCR-ready. However, there are two common causes for low amplification yield.

8.1 Incomplete removal of the Extraction Reagent. The Extraction Reagent contains a chaotrope that if carried over to the amplification reaction can result in a low yield. Perform the post-extraction step washes as described in Step 6.5.

8.2 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 filter. Dry the filter completely as described in Step 6.8.

9. PRODUCT SAFETY INFORMATION

Extraction Reagent

R11, 32
S7/9, 16

Wash Reagent

R11
S7/9, 16

Risk and Safety Phrases:

R11	Highly flammable.
R32	Contact with acid liberates very toxic gas.
S7/9	Keep container tightly closed and in a well-ventilated place.
S16	Keep away from source of ignition - No smoking.

Bio-Rad Phone Numbers: ***United States*** *1-800-224-6723* • ***Australia*** 61-2-9914-2800 • ***Austria*** 43-1-877-8901 • ***Belgium*** 32-9-385-5511 • ***Canada*** 905-712-2771 • ***China*** 86-10-6205-1850 • ***Denmark*** 45-39-17-99-47 • ***Finland*** 358-9-804-22-00 • ***France*** 33-1-4390-4690 • ***Germany*** 49-89-31884-0 • ***Hong Kong*** 852-2789-3300 • ***India*** 91-11-460-3676 • ***Israel*** 972-3-9514127 • ***Italy*** 39-02-216091 • ***Japan*** 81-3-5811-6290 • ***Korea*** 82-2-3473-4460 • ***Latin America*** 305-894-5950 • ***Mexico*** *52-5-514-0497* • ***The Netherlands*** 31-318-540666 • ***New Zealand*** 64-9-443-3099 • ***Russia*** 7-095-4585822 • ***Singapore*** 65-272-9877 • ***Spain*** 34-1-661-7085 • ***Sweden*** 46-8-627-50-00 • ***Switzerland*** 41-1-809-55-55 • ***United Kingdom*** *44-181-328-2000*

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