

PRONTO[®] DNA EXTRACTION KIT

For the extraction of DNA from human whole blood (EDTA)

Intended for use as an accessory to PRONTO[®] kits for mutation detection

Instructions for Use

REF 9925

48 Tests



MATERIALS PROVIDED

Solution A1 x bottle (100 mL)
Solution B11 x bottle (3.2 mL)
Solution B21 x vial (400 µL)

STORAGE OF REAGENTS

This kit should be stored at 4-8°C.
Minimize the time it spends at room temperature.

INSTRUMENTS REQUIRED

- Micro-centrifuge for 1.5 mL microtubes
- A 65°C - 95°C water bath or heat block
- Vortex mixer
- Pipettes (50-200 µL & 200-1000 µL)
- Vacuum aspirator (optional)

PROCEDURE

1. **Collect** blood (200 µL or more) in an EDTA-containing tube. Keep at 4-8°C up to 1 week or freeze at -20°C. **If you are using frozen blood samples, thaw them slowly immediately prior to use, at room temperature or at 4°C.**
2. **Agitate** the blood samples for 15 minutes immediately before use.
3. In a microtube, mix 200 µL blood with 600 µL of Solution A; vortex briefly, then centrifuge for 20 seconds at 13,000 RPM.
4. **Aspirate** all the liquid (leave behind about 10 µL); **avoid disturbing the pellet.**

5. **Re-suspend** the pellet by pipetting with 100 µL Solution A.
 - **Add** 500 µL more of Solution A.
 - **Vortex** briefly.
 - **Centrifuge** for 20 seconds at 13,000 RPM.
6. Carefully **aspirate** all the liquid; **avoid disturbing the pellet**.
7. **Repeat** steps 5 and 6. A white pellet should be visible.
8. **Prepare** Solution B by combining DNA Extraction Solutions B1 and B2. For every blood sample tested, mix 45 µL Solution B1 with 5 µL of solution B2. DNA Extraction Solution B should be prepared just before use.
9. **Add** the 50 µL of Solution B to the pellet and re-suspend it by pipetting
10. **Incubate** at 65°C for 1 hour.
11. Briefly **spin** down.
12. **Take** the tubes out of the water bath / heat block. Puncture a hole in the lid of every tube or use a screw cap tube.
13. **Incubate** the tubes for 10 min at 95°C.
14. Briefly **spin** down.
15. The DNA is ready for amplification. It may be kept for up to 6 months at 4°C.

WARNINGS AND PRECAUTIONS

It is recommended to use two separate sets of pipettes: one for extracting DNA and setting up the amplification reactions and the other for post-amplification handling of samples.

Use sterile tips throughout the procedure. Pipette tips with a filter are helpful in lowering the risk of sample cross- contamination and product contamination, especially when extracting DNA and setting up amplification reactions.

Potentially Infectious Material.

Universal Precautions should be taken when handling material of human origin, such as patient specimens.

All samples should be considered potentially infectious. Handling of samples, their use, storage and disposal should be in accordance with procedures defined by the applicable national or local biohazard safety guidelines or regulations.

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 **To prevent evaporation out of the punctured hole during storage it is recommended to transfer the sample to a new microtube.**