Instructions for Use

Version: 1.0.1
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Blood Genomic DNA Kit

Catalog No.: abx098868

Size: 50 rxns

Storage: Store at room temperature (15-25 °C) for up to one year. Keep dry.

Introduction

Abbexa's Blood Genomic DNA Kit uses magnetic beads to isolate genomic DNA from animal blood samples. The beads included with the kit are coated with a substrate that can bind DNA in the presence of a specially formulated buffer. A magnetic field retains these beads while contaminants and enzyme inhibitors, such as proteins and divalent cations, are washed away, allowing purified DNA to be eluted without the need for centrifugation. The isolated genomic DNA can be used in downstream applications and is suitable for PCR analysis, sequencing, enzyme digestion, and Southern blotting procedures.

Kit Components

Reagent	50 rxns
Binding Buffer	18 ml
Clean Buffer	50 ml
Wash Buffer	12 ml
Elution Buffer	10 ml
Proteinase K (20 mg/ml)	1 ml
Proteinase K	1 ml
Magnetic Beads	800 µl
Magnetic Stand (16 holes)	1 each

Material Required But Not Provided

- High-precision pipette and sterile pipette tips
- Microcentrifuge tubes
- Vortex mixer, inverter or sonicating water bath
- Absolute (100%) ethanol
- Absolute (100%) isopropanol

Reagent Preparation

- **Clean Buffer:** Dilute the Clean Buffer with absolute (100%) ethanol to a ratio of 1:1 (i.e. to the full 50 ml of Clean Buffer, add 50 ml absolute ethanol).
- **Wash Buffer:** Dilute the Wash Buffer with absolute (100%) ethanol to a ratio of 1:4 (i.e. to the full 12 ml of Wash Buffer, add 48 ml absolute ethanol).
- **Magnetic Beads**: Mix thoroughly by vortexing before use.

Sample Storage and Preparation

- Use 50 µl - 250 µl of whole blood. Thaw fully before use.
- **Short term storage**: Store at 2°C - 8°C for up to one week.
- **Long term storage**: Store at -80°C.

Assay Procedure

1. Add sample to a 1.5 ml microcentrifuge tube.
2. Add 300 µl of Binding Buffer and 20 µl of Proteinase K into the microcentrifuge tube. Mix well by vortexing.
3. Incubate at room temperature for 10 minutes, and vortex at least once during incubation.
4. Add 450 µl of 100% isopropanol to the microcentrifuge tube. Mix by vortexing for 10 seconds. Add 15 µl of vortexed Magnetic Beads into the microcentrifuge tube.
5. Vortex the microcentrifuge tube for 1 minute. Incubate at room temperature for 3 minutes.
6. Repeat Step 5 three times.
7. Place the microcentrifuge tube onto the magnetic stand until the Magnetic Beads have pelleted against the magnet. Remove as much supernatant as possible, taking care not to disturb the beads.
8. Remove the microcentrifuge tube from the stand, and add 800 µl of working Clean Buffer (diluted with absolute ethanol, see Reagent Preparation). Vortex for 2 minutes. Place the tube back onto the stand, and remove the supernatant as in Step 7.
9. Repeat Step 8.
10. Remove the microcentrifuge tube from the stand, and add 500 µl of working Wash Buffer (diluted with absolute ethanol, see Reagent Preparation). Vortex for 2 minutes. Place the tube back onto the stand, and remove the supernatant as in Step 7.
11. Repeat Step 10.
12. Uncap the microcentrifuge tube and allow the Magnetic Beads to air-dry, leaving the tube on the magnetic stand.
13. Remove the microcentrifuge tube from the magnetic stand, and add 100-200 µl of Elution Buffer. Mix gently by pipetting up and down several times until the beads are resuspended. Incubate at 56°C for 10 minutes. Mix gently by pipetting at least once during incubation.
14. Place the microcentrifuge tubes onto the magnetic stand, and separate beads as in Step 7. Carefully transfer the supernatant into a clean 1.5 ml tube. Avoid collecting any beads during this stage. Store the purified DNA at -20°C.

Notes

- All steps should be carried out at room temperature.
- It is recommended to use fresh samples where possible, and to avoid repeated freeze/thaw cycles.
- If a precipitate is observed in any of the buffer tubes, warm the tube to room temperature or to 37 °C until the precipitate has fully dissolved.
- Use sterile tubes and pipette tips to avoid DNase contamination.