Instructions for Use

Version: 1.0.2
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Genomic DNA Extraction Kit

Catalog No.: abx294005

Size: 50 tests / 200 tests

Storage: Store at room temperature (15-25 °C). Keep dry. For long-term storage, store at 2-8 °C.

Introduction

Abbexa's Genomic DNA Extraction Kit uses silica membrane technology to isolate genomic DNA from a variety of different samples. The spin columns included with the kit contain a silica membrane which can bind DNA in optimal salt and pH conditions. Contaminants and enzyme inhibitors such as proteins and divalent cations are removed by centrifugation, and purified DNA is eluted in low-salt buffer or water. The isolated genomic DNA can be used in downstream applications and is suitable for PCR analysis, restriction analysis, southern blotting, and use in cDNA libraries.

Kit Components

Reagent	50 tests	200 tests
GA Buffer	15 ml	50 ml
GB Buffer	15 ml	50 ml
GD Buffer	13 ml	52 ml
PW Buffer	15 ml	50 ml
TE Buffer	15 ml	60 ml
Proteinase K	1 ml	4 × 1 ml
Spin Columns	50	200
Collection Tubes (2 ml)	50	200

Material Required But Not Provided

- High-precision pipette and sterile pipette tips
- Centrifuge or microcentrifuge
- Microcentrifuge tubes
- Vortex mixer, inverter or sonicating water bath
- Incubator or heated water bath
- Absolute (100%) ethanol
- RNase A (100 mg/ml)
- Red Cell Lysis Buffer
- Distilled water (pH 7.0-8.5)

Yield

Sample	DNA Yield
Mammalian whole blood (100-400 µl)	3-10 µg
Avian or amphibian whole blood (5-20 µl)	5-40 µg
Cultured cells (10^6-10^7 cells)	5-30 µg
Animal tissue (30 mg)	10-30 µg

Reagent Preparation

- **Working GD Buffer solution:** Dilute the GD Buffer with absolute (100%) ethanol to a ratio of 13:17 (i.e. to 13 ml of GD Buffer, add 17 ml of absolute (100%) ethanol to form 30 ml of Working GD Buffer solution).
- **Working PW Buffer solution:** Dilute the PW Buffer with absolute (100%) ethanol to a ratio of 1:4 (i.e. to 15 ml of PW buffer, add 60 ml of absolute (100%) ethanol to form 75 ml of Working PW Buffer solution).

Sample Preparation

- **Mammalian blood:** Use 200 µl of fresh, frozen or anticoagulant-added blood as the sample volume. If the sample is less than 200 µl, add GA Buffer until the volume is 200 µl.

  Note: if the blood volume is between 300-1000 µl, add Red Cell Lysis Buffer to a ratio of 1:3 (e.g. to 300 µl blood, add 900 µl Red Cell Lysis Buffer). Close the cap on the tube and ensure it is secure, then invert the tube and allow to stand at room temperature for 5 minutes. Centrifuge for 1 minute at 10,000 RPM. Discard the supernatant. Resuspend the pellet in 200 µl of GA Buffer and mix thoroughly using a vortex mixer.
- **Avian blood, amphibian blood, and blood cells containing a nucleus:** Take 5-20 µl of sample and add GA Buffer until the volume is 200 µl.
- **Cells:** Adherent cells should be treated to obtain a cell suspension. Centrifuge the cells for 1 minute at 10,000 RPM. Discard the supernatant. Resuspend the cell pellet in 200 µl of GA Buffer.
- **Animal tissue:** Treat tissue to obtain a cell suspension (for spleen tissue, use < 10 mg tissue). Centrifuge the cells for 1 minute at 10,000 RPM. Discard the supernatant. Resuspend the cell pellet in 200 µl of GA Buffer.

Assay Procedure

1. To a microcentrifuge tube, add sample (see Sample Preparation above).
2. If RNA-free genomic DNA is required, add 4 µl of RNase A (100 mg/ml, not included with this kit), mix thoroughly using a vortex mixer for 15 seconds, then allow to stand for 5 minutes at room temperature; otherwise skip this step.
3. Add 20 µl of Proteinase K to the tube. Mix by vortexing. For tissue samples, incubate at 56 °C until the tissue is completely lysed. The lysis time will depend on the type of tissue used. Lysis usually takes 1-3 hours for most samples (though samples such as rat tail will need to be lysed overnight). Samples should be inverted 2-3 times every hour or placed in a sonicated water bath.
4. Add 200 µl of GB Buffer to the tube. Mix thoroughly using a vortex mixer, then incubate at 70 °C to yield a clear solution. Briefly centrifuge the tube to ensure complete recovery of vial contents.
   Note: white precipitates may form when GB Buffer is added. They do not interfere with the assay and will dissolve when incubated at 70 °C. Precipitates that do not dissolve after incubation indicate incomplete cell lysis, which may result in low yield and/or impurities. If samples have not been stored appropriately or blood samples with volume < 200 µl have not been treated with Red Cell Lysis Buffer, the color may be dark brown after incubation.
5. Add 200 µl of absolute ethanol to the tube. Mix thoroughly using a vortex mixer for 15 seconds. Flocculent precipitates may be observed. Briefly centrifuge the tube to ensure complete recovery of vial contents.
6. Transfer the contents of the tube to a spin column (in a 2 ml collection tube). Centrifuge for 30 seconds at 12,000 RPM, then discard the flow-through. Place the spin column back into the collection tube.
7. Add 500 µl of Working GD Buffer solution (with ethanol) to the spin column. Centrifuge for 30 seconds at 12,000 RPM, then discard the flow-through. Place the spin column back into the collection tube.
8. Add 600 µl of Working PW Buffer solution (with ethanol) to the spin column. Centrifuge for 30 seconds at 12,000 RPM, then discard the flow-through. Place the spin column back into the collection tube. Repeat this step once more for a total of two times.
9. Centrifuge at 12,000 RPM for 2 minutes to remove any residual buffer solution. Air-dry the spin column at room temperature for several minutes.

10. Place the spin column into a sterile microcentrifuge tube. Add 50-200 µl of TE Buffer or distilled water (pH 7.0-8.5) to the center of the column. Allow to stand at room temperature for 2-5 minutes.
11. Centrifuge for 2 minutes at 12,000 RPM and collect the liquid, which contains the eluted DNA. The purified DNA can be stored at -20 °C for long-term storage. To recover more genomic DNA, add the liquid obtained to a spin column and allow to stand for 2 minutes. Centrifuge for 2 minutes at 12,000 RPM and collect the liquid.

Notes

- All centrifugation 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.