Protocol

MBead Blood/ Cell Genomic DNA Kit

Cat No. PDM06-0100(S) Size: 100 Reactions



Description

This MBead Blood/ Cell Genomic DNA Kit was designed specifically for isolating the genomic DNA from the blood samples. Its unique buffer system will efficiently lyse cells and degrade proteins, allowing for the DNA to be easily bound by the surface of the magnetic beads. The RNA and other non-specific binding particles are removed with a wash buffer, and the genomic DNA is then released into the Release Buffer. The genomic DNA can be purified manually within 10~15 minutes (using most magnetic separators) or the kit can be easily adapted to satisfy most automated nucleic acid purification systems.

Specifications

- Sample: Up to 300 µl of the blood/ cell
- Format: Manual or automated genomic DNA isolation
- Operation time: Within 10~15 minutes (manual)
- Applications: Restriction Enzyme Digestion, Southern Blotting, PCR, qPCR and RT-PCR assays
- Storage: Room temperature

Kit Contents

Contents	PDM06-0100	PDM06-0100S
Magnetic Bead	2 ml	80 ul
Lysis Buffer	30 ml	1.5 ml
Wash Buffer	80 ml	4 ml
Release Buffer	20 ml	1 ml

Required Materials

- ➤ Absolute EtOH
- ➤ 1.5 ml microcentrifuge tubes
- Magnetic separator
- > Water bath / Dry bath

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Step 1 Lysis

- 1. Transfer up to 300 μ l of the blood/ cell into a 1.5 ml microcentrifuge tube and add 300 μ l of the Lysis Buffer.
- 2. Mix well and incubate 65°C for 5 minutes. During this time, pre-heat the Release Buffer to 65°C for the Step 4.
- 3. Add 300 µl of the absolute EtOH to the lysate and mix well.

Step 2 DNA Binding

- 1. Add 20 µl of the Magnetic Beads. Mix well by gently shaking for 3 minutes.
- 2. Place the tube in a magnetic separator for 30 seconds.
- 3. Remove the solution (If the mixture becomes viscous, increase magnetic bead separation time).

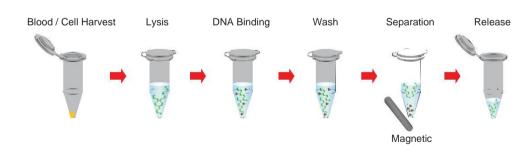
Step 3 Wash

- 1. Add 800 μ I of the Wash Buffer and mix well (Following the wash, the mixture will no longer be viscous).
- 2. Place the tube in a magnetic separator for 30 seconds. Remove the solution.

Step 4 Release

- 1. Add 200 µl of the Release Buffer (pre-heated to 65°C) and mix well.
- 2. Incubate for 3 minutes at 65°C (During incubation, shake the tube vigorously every minute).
- 3. Place the tube in a magnetic separator for 1 minute.
- 4. Carefully transfer ONLY the clean portion of the solution to a clean tube.

NOTE: Be sure and allow the magnetic beads to disperse completely during the binding, wash and elution steps.







Protocol

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when purifying the genomic DNA with the kit.

Problem	Cause	Solution
DNA is sheared or degraded	Lysate mixed too vigorously	Use the appropriate pipette tip set for the required volume, lower it under the reading line of the solution to mix the sample, pipet up and down gently to mix.
	DNase contamination	Maintain a sterile environment while working (e.g. wear gloves and use DNase-free reagents).
RNA containment	Incomplete removal of the RNase	RNase A treatment
Low yields of gDNA	Incomplete lysis and homogenization	Complete lysis. Use the appropriate method for the lysate preparation based on the amount of starting materials.
	Incorrect handling of Magnetic Beads	Vortex the tube containing the magnetic beads to fully resuspend the beads before adding them to your sample.
	Incorrect elution conditions	Add the Release Buffer (50~100µl) and incubate for 3 min at 65°C.
	The quality of the starting material may not be optimal	Use the fresh sample and process immediately after collection, or freeze the sample at -80°C or in the liquid nitrogen.
High background on UV measurement	Residual beads released	Repeat the magnetic separation and transfer the eluate to a clean tube

Caution

- > During the operation, always wear a lab coat, disposable gloves, and protective equipment.
- > Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.

