MBead Buffy Coat Genomic DNA Kit

Cat No. PDM01-0100(S)
Size: 100 Reactions

Description

This magnetic bead genomic DNA purification kit was designed specifically for isolating the genomic DNA from the Buffy Coat. 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 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.

Application

- Sample: Up to 300 µl of the buffy coat
- Operation time: 10~15 minutes (manual)
- Applications: Restriction Enzyme Digestion, Southern Blotting, PCR and qPCR assays
- Storage: Room temperature

Kit Contents

Contents	PDM01-0100	PDM01-0100S
Magnetic Bead	2 ml	80 µl
Lysis Buffer	30 ml	1.5 ml
Wash Buffer	80 ml	4 ml
Release Buffer	20 ml	1 ml

Required Materials

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

Protocol

Step 1 Lysis

1. Transfer up to 300 µl of the Buffy Coat into a 1.5 ml microcentrifuge tube and add 300 µl of the Lysis Buffer.
2. Mix well and incubate at 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 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)

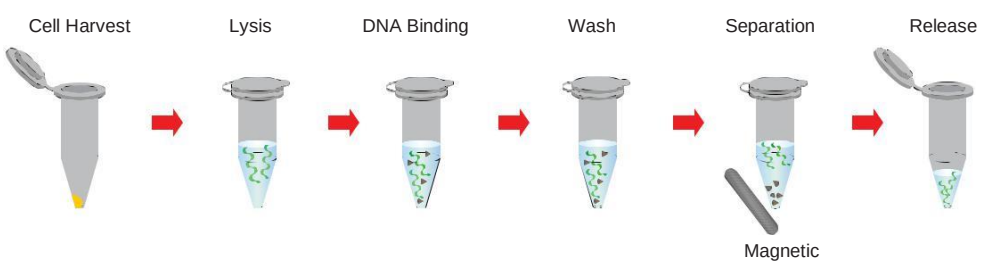
Step 3 Wash

1. Add 800 µl 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 the 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.



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.
	DNases 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 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

- Check buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- During 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.