MBead Tissue Genomic DNA Kit

Cat No. PDM02-0100
Size: 100 Reactions

Description

This MBead Tissue Genomic DNA Kit is designed specifically for isolating the genomic DNA from animal tissue 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 50 minutes (using most magnetic separators) or the kit can be easily adapted to satisfy most automated nucleic acid purification systems.

Specifications

- Sample: Up to 30mg of the animal tissue
- Format: Manual or automated genomic DNA isolation
- Operation time: Within 50 minutes (manual)
- Applications: Restriction Enzyme Digestion, Southern Blotting, PCR and qPCR assays
- Storage: Room temperature

Kit Contents

Contents	NA009-0100
Magnetic Bead	2 ml
Lysis Buffer	30 ml
Grind Buffer	25 ml
Wash Buffer	80 ml
Release Buffer	20 ml

Required Materials

- Absolute EtOH
- Magnetic separator
- 1.5 ml microcentrifuge tubes
- Proteinase K (10 mg / ml)
- Water bath/ Dry bath

MBead Tissue Genomic DNA Kit Protocol

Sample Preparation

1. Cut off up to 30 mg of the animal tissue and transfer it to a 1.5 ml microcentrifuge tube.
2. Add 200 µl of the Grind Buffer to the tube and continue to homogenize the sample tissue by grinding.

Step 1 Lysis

1. Add 20 µl of the Proteinase K (10 mg/ml) to the sample mixture and mix by vortex.
2. Incubate at 65°C for 30 minutes to lyse the sample. During the incubation, invert the tube every 5 minutes.
3. Centrifuge for 5 minutes at 5,000 x g.
4. Transfer the supernatant to a new 1.5 ml microcentrifuge tube and add 300 µl of the Lysis Buffer.
5. 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.
6. 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 the magnetic bead separation time).

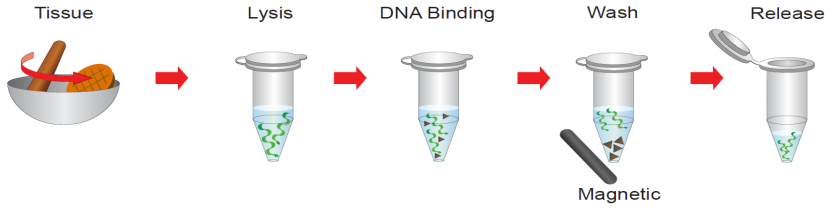
Step 3 Wash

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 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.
RNA contaminant	Incomplete removal of the RNAse	RNase A treatment
Low yields of gDNA	Incomplete lysis and homogenization	Complete lysis and homogenization. Grind completely. Use the appropriate method for the lysate preparation based on the amount of the starting materials. Decrease the amount of the starting material used. Cut the tissue samples into smaller pieces, and ensure the tissue is completely immersed in the Lysis Buffer to achieve the optimal lysis.
	Protein contaminant	Check that the Proteinase K has been added.
	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 the UV measurement	Residual beads released	Repeat the magnetic separation and transfer the eluate to a clean tube

Related Ordering Information

Cat. No.	Description	Size
MB101-0500	*Taq* DNA polymerase	500 U
MB200-0100	PCR SUPERMIX	100 RXNS
MB201-0100	Hot Start SUPERMIX	100 RXNS
MB25530-0025	Ultrapure Proteinase K	25 mg
AGT001-0500	AGAROSE Tablet, 0.5g	100 tab
LD001-1000	Novel Juice Supplied in 6X Loading Buffer	1 ml
DM003-R500	100 bp DNA Ladder H3 RTU	500 μl
DM010-R500	1 Kb DNA Ladder RTU	500 μl
DM013-R500	XLarge DNA Ladder RTU	500 μl
DN001000	100 mM dNTP Set	4x1 ml
DN001-0250	100 mM dNTP Set	4 x 250 μl
DN025-1000	2.5 mM dNTP Mix	1 ml
DN0010	10 mM dNTP Mix	1 ml

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.