TIANGEN®

Magnetic Animal Tissue Genomic DNA Kit

For genomic DNA purification from variety of animal tissues

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Magnetic Animal Tissue Genomic DNA Kit

Cat. no. 4992404/4992405/4992864

Kit Contents

Contents	4992404 (50 preps)	4992405 (200 preps)	4992864 (1000 preps)
Buffer GHA	12 ml	50 ml	5 × 50 ml
Buffer GHB	20 ml	80 ml	5 × 80 ml
Buffer GDA	25 ml	90 ml	5 × 90 ml
Buffer PWD	20 ml	2 × 40 ml	5 × 2 × 40 ml
Proteinase K	1 ml	4 × 1 ml	5 × 4 × 1 ml
MagAttract Suspension G	2 × 1 ml	6 × 1 ml	5 × 6 × 1 ml
Buffer TB	15 ml	60 ml	5 × 60 ml
Handbook	1	1	5

Required reagent

RNase A (100 mg/ml) (TIANGEN)

Storage

Magnetic Animal Tissue Genomic DNA Kit can be stored dry at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C before use.

Introduction

Magnetic Animal Tissue Genomic DNA Kit is designed for isolation of high-quality genomic DNA from animal tissues with unique magnetic particles technology and special buffer system. The unique magnetic beads have a strong affinity for nucleic acid under certain conditions, and when the conditions change, the magnetic beads will release the absorbed nucleic acid, so as to achieve the purpose of nucleic acid purification. The whole process does not involve phenol and chloroform, it is safe and convenient, and the extracted genomic DNA fragments are large, high purity, stable and reliable in quality, which is especially suitable for automated extraction of high-throughput workstation.

DNA purified by this kit is suitable for direct use in most downstream applications such as enzymatic reactions, PCR, real-time PCR, library preparation, Southern blotting, chip detection, high-throughput sequencing, etc.

Product Feature

Simple and fast: Samples can be lysed without incubating to obtain gDNA within 1 h.

High-throughput: The kit can be integrated with automatic instrument of pipetting method and magnetic bar method to carry out high-throughput extraction.

Safe: No toxic organic reagents such as phenol/chloroform are needed.

High purity: The gDNA obtained with high purity can be directly used for chip detection and high-throughput sequencing.

Important Notes Please read the notes before use.

1. The kit is suitable for DNA purification by manual operation or automated platform.
2. Supplied by user: 96-100% ethanol; Isopropanol
3. Repeated freezing and thawing should be avoided; otherwise it would reduce the DNA size and quantity.
4. If a precipitate has formed in Buffer GHB, warm buffer at 37°C until the precipitate has fully dissolved.
5. If RNA-free genomic DNA is required, RNase A (100 mg/ml) should be prepared by user.

Extraction Yield

Sample		Suggested sample weight	DNA yield (μg)
Rodent	Brain	25 mg	20-30
	Heart	25 mg	20-30
	Liver	25 mg	30-50
	Spleen	25 mg	50-70
	Lung	25 mg	50-70
	Kidney	25 mg	30-50
	Tail	Rat 0.3 cm Mouse 0.6 cm	50-80
Other	Muscle	50 mg	5-10
	Fish	50 mg	5-10
	Shrimp	50 mg	5-10
	Seashell	30 mg	30-50
Micro DNA	Swab	1	0.2-1
	Dried blood spot	3 pieces 3×3 mm	0.2-1

Protocol

Ensure that ethanol (96-100%) has been added into Buffer GDA and Buffer PWD before use as indicated on the tag.

(I) Manual Operation

1. Dissect 10 to 50 mg fresh tissue and transfer into a 1.5 ml microcentrifuge tube. Add 200 μl Buffer GHA and 20 μl Proteinase K, then use an electric homogenizer to grind the tissue completely for 10 sec.

   1) If tissue is not completely lysed, the sample can be incubated at 65°C until completely lysed.

   2) If the sample lysed completely, skip 65°C incubation step.

   3) Mouse tail can be incubated at 56°C overnight for complete lysing.

   Note: In order to speed up lysis, cut the tissue into small pieces. If there is still tissue debris after incubation, centrifuge at 12,000 rpm for 1 min is recommended and remove the deposit.

   If RNA-free genomic DNA is required, add 4 μl RNase A (100 mg/ml, should be prepared by user), and incubate for 10 min at room temperature (15-30°C).

2. Add 300 µl Buffer GHB, and mix completely by pipetting or vortex.
3. Heat at 75°C for 15 min, during which invert the sample tube 3 times.
4. Incubate at room temperature for 5 min.
5. Add 350 µl isopropanol and vortex for 10 sec.
6. Add 30 µl MagAttract Suspension G, vortex for 1 min. Incubate for 9 min at room temperature and vortex for 1 min per 3 min.

   Note: Vortex MagAttract Suspension G completely before adding to the sample.

   Recommended volume of the magnetic particles for Swab and Dried blood spot is 15 µl; for muscle tissue is 20 µl; for rat spleen is 30 µl.
7. Place the microcentrifuge tube to a magnetic separation device for 30 sec, allowing the magnetic particles to completely clear from the solution. Discard the supernatant carefully.
8. Add 700 µl Buffer GDA **(Ensure that ethanol (96-100%) has been added) and vortex for 30 sec.**
9. Place the microcentrifuge tube to the magnetic separation device for 30 sec, allowing the magnetic particles to completely clear from the solution. Discard the supernatant carefully.
10. Add 700 µl Buffer PWD **(Ensure that ethanol (96-100%) has been added) and vortex for 30 sec.**
11. Place the microcentrifuge tube to magnetic separation device for 30 sec, allowing the magnetic particles to completely clear from the solution. Discard the supernatant carefully.
12. Repeat step 10 and 11.Clear the solution as possible.
13. Place microcentrifuge tube on the magnetic separation device for 10-15 min to air dry.

    Note: Residual ethanol may inhibit subsequent enzymatic reactions. Please ensure the residual ethanol is removed completely. However, overdrying should be avoided, since over-dried DNA is difficult to dissolve.
14. Remove the microcentrifuge tube from the magnetic separation device. Add 100-200 µl Buffer TB to elute DNA. Mix by pipetting or vortex and incubate at 56°C for 10 min, during which invert the tube 3 times.
15. Place the microcentrifuge tube on the magnetic separation device for 2 min until all the magnetic particles are cleared from the solution. Transfer the supernatant containing purified DNA to a new collection microcentrifuge tube and store.

(II) Automated Purification System

Before Starting

1. This system can be easily adapted to a variety of automated platforms, such as Hamilton Microlab STAR, Beckman Coulter Biomek® FX, Capitalbio LabKeeper and so on.
2. The samples were treated the same as manual protocol. Transfer 200 µl tissue lysate into a 96 deep-well plate.
3. Mix the MagAttract Suspension G and isopropanol as 3:7 (v/v). Add 100 µl mixture to each sample.
4. Hamilton Microlab STAR provides a 2 ml microcentrifuge tube position, so it's unnecessary to dilute the magnetic particles with isopropanol. Add 350 µl isopropanol. Each tube could be loaded with about 1 ml magnetic particles. Pipette up 5 times and add 30 µl magnetic particles to each well. Close the lid after pipetting.
5. Spleen, liver and kidney have abundant genomic DNA. It is suggested to pipette 5 times after adding isopropanol, then mixed with magnetic particles completely to avoid aggregation and insufficient washing.
6. There is deviation between setting temperature and real temperature of 96 deep-well plate. It is suggested to set 10 °C higher than temperature given in the manual.

Protocol

1. Transfer 200 µl tissue lysate into a 96 deep-well plate (provided by user).
2. Add 300 µl Buffer GHB to each well, pipette 6 times.
3. Place the plate at 75°C for 15 min, then shake to mix completely.
4. Set the heating block to 25°C and shake for 5 min.
5. Add 270 µl isopropanol to each well, pipette 6 times and shake 5 min.
6. Add 100 µl MagAttract Suspension G to each well, pipette 6 times and shake for 10 min.
7. Place the plate to a magnetic separation device for 2 min, allowing the magnetic particles to completely clear from the solution. Discard the supernatant carefully.
8. Remove the magnetic separation device. Add 100 µl Buffer GDA and shake for 2 min. Add 600 µl Buffer GDA pipette up 6 times and shake for 2 min.
9. Place the plate to magnetic separation device for 30 sec, and then discard the supernatant carefully, after the magnetic particles absorbed completely.

Note: If need to increase the purity of genomic DNA, could repeat step 8 and 9.

10. Remove the magnetic separation device. Add 100 µl Buffer PWD and shake for 1 min. Add 600 µl Buffer PWD pipette up 6 times and shake for 2 min.
11. Place the plate to magnetic separation device for 30 sec, and then discard the supernatant carefully.
12. Repeat step 10 and 11. Clear the solution as possible.
13. Place plate on the magnetic separation device at 37°C for 5 min to air dry.
14. Remove the magnetic separation device. Add 100-200 µl Buffer TB and shake at 65°for 10 min.
15. Place the plate on the magnetic separation device for 2 min until all the magnetic particles are cleared from the solution. Transfer the supernatant containing purified DNA to a new collection plate and store.

(III) Magnetic rods System

Before Starting

1. This system can be easily adapted to a variety of automated platforms, such as Thermo KingFisher Flex.
2. The samples were treated the same as manual protocol.Transfer lysate into a 96 deep-well plate.
3. Add 300 µl Buffer GHB, 700 µl Buffer GDA, 700 µl Buffer PWD and 100-200 µl Buffer TB to the suitable position of the 96 deep-well plate. Add 30 µl MagAttract Suspension G to 700 µl Buffer GDA.
4. The Magnetic rods System can refer to the protocol of Automated Pipetting System. However, it should “pause” and add isopropanol after the lysis step.

Protocol

1. Transfer 200 µl lysate into a 96 deep-well plate.
2. Place the plate at 75°C for 15 min. Pipette up with middle and high speed in turn.
3. During the Pause Step add 350 µl isopropanol in each well. Pipette in high speed for 5 min.
4. Pipette in high speed for 1 min to make magnetic particles dispersed.
5. Move the magnetic rod in wells 3 times (20 sec per time) to collect magnetic particles.

6. Transfer particles to wells with tissue lysate and Buffer GHB, release particles and pipette up with middle and high speed in turn for 10 min.
7. Move the magnetic rod in wells 3 times (20 sec per time) to collect magnetic particles.
8. Transfer particles to wells with Buffer GDA. Release particles and pipette up with high speed for 3 min.
9. Move the magnetic rod in wells 3 times (20 sec per time) to collect magnetic particles.
10. Transfer particles to other wells with Buffer GDA. Release particles and pipette up with high speed for 3 min.
11. Move the magnetic rod in wells 3 times (20 sec per time) to collect magnetic particles.
12. Transfer particles to wells with Buffer PWD. Release particles and pipette up with high speed for 3 min.
13. Move the magnetic rod in wells 3 times (20 sec per time) to collect magnetic particles.
14. Transfer particles to other wells with Buffer PWD. Release particles and pipette up with high speed for 3 min.
15. Move the magnetic rod in wells 3 times (20 sec per time) to collect magnetic particles.
16. Keep the particles on the magnetic rod for 5 min to air dry.
17. Transfer particles to wells with Buffer TB. Incubate at 75°C and pipette up with high speed for 10 min.
18. Move the magnetic rod in wells 3 times (20 sec per time) to collect magnetic particles.
19. Transfer the particles to wells with Buffer PWD and abandon.
20. Transfer the supernatant containing purified DNA to a new collection plate and store.

DNA concentration and purity detection

The obtained DNA fragment is related to sample storage time and shear force during operation. The concentration and purity of DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer.

DNA should have a significant absorption peak at OD_{260}. If the OD_{260} value is 1, then it is equivalent to about 50 μg/ml double-strand DNA and 40μg/ml single-strand DNA.

The OD_{260}/OD_{280} ratio should be 1.7-1.9. If it is not the elution buffer but ddH_2O is used, the ratio will be low, because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.