INSTRUCTIONS

Magnetic Genomic DNA Extraction Kit

Catalog Number： DE01001 DE01002 DE01003 DE0196B

AvanBio Inc.

239 New Road, Suite A-107
Parsippany, NJ 07054
U.S.A.

Phone: +1 (800) 278-9865
Fax: +1 (855) 378-6845
Email: support@avanbio.com

【Introduction】

EmerTher Genomic Magnetic DNA Extraction Kit is used to extract DNA from plasma, cells, saliva and other aqueous biological samples.

The Kit contains superparamagnetic nanoparticles which are efficiently bound to nucleic acids and a safe and eco-friendly extraction system. The magnetic beads are coated through a unique process, enabling strong binding with nucleic acids. The experiment procedure is simple and efficient: 1) Add sample to a lysis-binding solution to enable cell lysis, DNA release and DNA binding to magnetic beads; 2) apply magnetic force enabling easy wash of the beads with buffers; 3) elution of DNA from the beads using water or TE buffer.

Extracted DNA can be directly used for gene sequencing, digestion, PCR amplification, library construction, Southern hybridization and other subsequent experiments.

【Product Features】

High-throughput: fast extraction of genomic DNA from aqueous samples

Eco-friendly: do not contain hazardous organic solvents, such as phenol or chloroform

Automation: no liquid-liquid mixing or centrifugation process; therefore, the whole procedure can be easily automated

【Components】

Catalog No.	***DE01001 (20 preps)***	***DE01002 (100 preps)***	***DE01003 (500 preps)***	***DE0196B (96 preps)***
Format	Bottles	Bottles	Bottles	96-well standard plates
PK Dissolving Solution	0.4 ml	2 ml	10 ml	2 ml
Proteinase K	8 mg	40 mg	200 mg	40 mg
Magnetic Bead Suspension	1 ml	3 ml	15 ml	Pre-packed with magnetic beads and extraction reagents
Lysis-binding Buffer	15 ml	70 ml	180 ml × 2	
Wash Solution I	20 ml	80 ml	200 ml × 2	
Wash Solution II	20 ml	80 ml	200 ml × 2	
Wash Solution III	75% ethanol in water (prepared by user)			
Elution Solution	ddH_2O or TE buffer (pH7.5-8.5) (prepared by user)			

【Storage and Expiration】

1. The kit is shipped at room temperature. Upon receipt, please store proteinase K and PK Dissolving Solution at -20°C;
2. Prior to use, prepare a proteinase K solution by adding PK Dissolving Solution to the bottle containing proteinase K, mix well and store the solution at -20°C;
3. The remaining reagents can be stored at room temperature for 18 months.

【Companion Device】

A magnetic separation rack and a rotary mixer (for manual extraction); or an automatic magnetic particle processor

【Experiment Procedure】

1. Mix proteinase K and PK Dissolving Solution to prepare a proteinase K solution (20 mg/ml);
2. Transfer 200-300 µl of aqueous sample into a 2 ml centrifuge tube. Add 20 µl proteinase K solution , 700µl Lysis-binding Buffer, and 30 µl bead suspension; place the sample on a rotary mixer and mix thoroughly for 30 min, DNA is released from cells and binds to the beads.
3. Place the tube on a magnetic separation rack, so beads are pulled to the side of the tube. Discard supernatant.
4. Add 800 µl Wash Solution I and re-suspend the beads. Transfer the solution containing the beads to a new centrifuge tube, gently invert the tube for 10 min to wash the beads. Place the tube back to the magnetic separation rack, discard supernatant.
5. Add 800 µl Wash Solution II to wash the beads for 3 min. Place the tube back to the magnetic separation rack, discard supernatant.
6. Add 800 µl Wash Solution III to wash the beads for 3 min. Place the tube back to the magnetic separation rack, discard supernatant.
7. Add 800 µl Wash Solution III again to wash the beads for 3 min. Place the tube back to the magnetic separation rack, discard supernatant.
8. Let the beads stand for 3-5 min to remove residual ethanol.
9. Add 100 µl Elution Solution and mix with beads for 10 min at 60°C in a water bath to release DNA from the beads. The volume of Elution Solution can be reduced if a higher concentration of DNA is desired.
10. Place the tube back to the magnetic separation rack and transfer supernatant containing DNA to a clean centrifuge tube for subsequent testing or storage at -20°C.

[Steps 2-10 can be completed on an automatic magnetic bead processor/liquid handler.]

Note:

a. Instruction for pre-treatment of cultured cells:
   Wash cells adherent to a 3.5cm culture plate (about 10^6 cells) with PBS. Remove PBS and add 1ml of 2% Triton X-100 (If a different type of culture plate is used, the volume of Triton X-100 may increase to cover the surface area of the culture plate), pipette several times, and transfer the mixture of cells and the Triton X-100 solution to a 1.5ml centrifuge tube. The remaining experimental procedure is the same as described above.

【Additional Information】

1. The Lysis-binding Buffer may precipitate after long-term storage at low temperature. If the temperature is below 15°C, please warm the solutions at 37°C in a water bath until they turn to clear solutions before use.
2. The Lysis-binding Buffer contains protein denaturing agents, thus it is corrosive. Please handle with care. If the solution is accidentally splashed on skin, please wash with plenty of water.
3. The viscosity of some samples (e.g.blood) is high after lysis, thus sufficient time (recommend 1-2min) should be given to enrich beads for these samples.
4. Remove Wash Solution III completely before adding Elution Solution. Let beads stand for 3-5 min to remove residual ethanol. Too much ethanol residual may affect subsequent tests. But if the sample is too dry, it may be difficult for DNA to be eluted from beads and the yield may be reduced.
5. The volume of the elution solution can be reduced to increase DNA concentration in the eluent, but may decrease the total extraction amount. In general, using the same total elution volume with repeated elution processes (a smaller elution volume is used at each time) can increase the elution efficiency and the total

extraction amount.

6. For extracting DNA from blood, it is recommended to increase the concentration of the proteinase K solution to 40 mg/ml of and keep other experimental conditions unchanged.
7. Some components in cell culture medium may interfere with the extraction system. In this case, it is recommended to spin down the cells and add 0.5 ml of 2% Triton X-100 to cell pellet. Pipette several times and use 200-300 µl of the cell mixture to start the experiemnt.
8. The amount of liquid sample used in an experiment is 10-400 µl, most preferably 200-300 µl.

【Trouble-shooting】

1. The recovery of genomic DNA is low
   A. Sample is not mixed well. Please shake and mix the sample thoroughly before sampling.
   B. The amount of beads added is insufficient. Please shake and mix the bead suspension well before adding the beads to a sample.
   C. The binding of DNA to the beads is insufficient. Please gently shake or invert the sample mixture during the binding process to ensure sufficient binding of DNA to the beads.
   D. Sample is not preserved in accordance with the requirements, leading to degradation of genomic DNA.
2. Extracted DNA has dispersed binds, indicating fragmentation of DNA
   A. Sample is stored for too long or is not stored in a continuously frozen state. Please use fresh and properly preserved samples.
   B. Operation is too harsh, thus DNA is mechanically fragmented. Please strictly follow the instructions.
3. Extracted DNA sample is impure, suppressing or interfering with the subsequent test
   A. After cell lysis and the addition of Wash Solution I, the sample solution is not transferred to a new centrifuge tube. Some impurities remain at the inner wall of the tube and may elute in the final solution. Please change to a new centrifuge tube after the addition of Wash Solution I.
   B. Beads are not completely dried. Residual ethanol is still in the sample and interferes with subsequent test. Try to remove any liquid in the centrifuge tube completely after the last wash.
   C. Let the beads stand for 3-5min before the addition of Elution Solution to ensure complete evaporation of ethanol.

Automation procedure and setup for DE0196B

1. Setup for pre-packed 96 deep-well plates (extraction of 16 samples per run)

Columns 1,7 	**Columns 2,8** 	**Columns 3,9** 	**Columns 4,10** 	**Columns 5,11** 	**Columns 6,12** 
700 µl Lysis-binding Buffer, 200 µl aqueous sample, 20 µl proteinase K solution	800 µl Wash Solution I; 30 µl magnetic beads	800 µl Wash Solution II	800 µl Wash Solution III	800 µl Wash Solution III	100 µl Elution Solution

2. Add sample and 20 µl of proteinase K to each well (columns 1 and 7), which are prefilled with Lysis-binding Buffer (If room temperature is below 15°C, please warm the plate at 37°C for 20 min to ensure complete dissolution of any precipitates before use.).
3. Install two sets of tip combs (8 tip combs per set), select the automation procedure listed in the below table and start to extract nucleic acids.

Sequence	**Column No.**	**Function**	**Mixing time (sec.)**	**Magnetic adsorption time (sec.)**	**Waiting time (sec.)**	**Volume (µl)**	**Mixing speed**	**Temp (℃)**
1	1	Lysis	900	0	0	900	fast	50
2	2	Transfer Beads	30	20	0	800	medium	Room Temp
3	1	Binding	600	30	0	900	fast	50
4	2	Wash 1	600	20	0	800	fast	Room Temp
5	3	Wash 2	240	20	0	800	fast	Room Temp
6	4	Wash 3	180	20	0	800	fast	Room Temp
7	5	Wash 4	180	30	60	800	fast	Room Temp
8	6	Elution	420	60	0	100	fast	70
9	4	Move beads	30	0	0	800	fast	Room Temp

4. The elution solutions containing purified nucleic acids are transferred out from the columns 6 and 12, and used for subsequent testing or storage at -20°C.