Tissue&cell Genomic DNA Midi Purification Kit

Description:

The Tissue & Cell Genomic DNA Midi Purification Kit provides a rapid, simple and effective approach to isolate the genomic DNA from various animal tissues up to 100mg and culture cells up to $2x10^7$. The process is based on a spin column format; the procedure involves cell lysis with proteinase K digestion, nucleic acids absorption and DNA elution. There is no requirement for phenol/chloroform extraction or alcohol precipitation. Typical yield ranges from 100-120μg. DNA purified with this kit is suitable for various applications, including PCR, restriction enzyme digestion, cloning, dot blot analysis, etc.

Characteristics:

- Fast spin-column
- Centrifugation-based method.
- No phenol/chloroform extraction and ethanol precipitation required.
- Consistent, high yields
- Complete removal of contaminants and inhibitors
- Operation time: < 60 minutes
- Wide range sample from plant tissue

Product Name	Size	Cat.No
Tissue&cell Genomic DNA Midi Purification Kit	25 Reactions	Bio-GTD-25

Applications

- PCR
- Southern Blotting
- Medicolegal Analysis
- Real-Time PCR,
- AFLP, RFLP, PAPD.

Storage Condition:

store at RT

Components of the kit :

	GTD-25
1. Lysis Buffer	30 ml
2. Binding Buffer	30 ml
3. Proteinase K solution	10 mg/mL , 2.7mL
4. Wash Buffer I	30 ml (add 165ml of Ethanol before use)
5. Wash Buffer II	30 ml
6. Elution Buffer	30 ml
7. Midi Spin column	25 pcs
8. Midi Collection tube	25 pcs

Before beginning this procedure :.

- Store the Proteinase K at -20℃. store all other kit components at RT
- RNA-free genomic DNA is required, prepared 100mg/ml RNase A **(not include in the kit)** with H_2O.
- Turn on the water baths or heat blocks at 56℃ and 70℃.

General Procedure :

Materials to be supplied by the user:

- For tissue grinding : Small homogenizer (fisher Tissue Tearor. Polytron or Turax). Alternatively, mortar and pestle.
- DNase-free RNaseA
- Trypsin (for adherent tissue culture cells only).
- PBS buffer or TE buffer (culture cells only)
- Ethanol

Protocol for isolation of genomic DNA from tissue & Cell

1. **a. Tissue culture cells :** (< 2 X 10^7)

*** Do not use too much sample, which will clog the column and obtain lower yield and quality.**

(I)Harvest the cells (for adherent cells, trypsinize the cells before harvesting) and transfer to 50ml centrifuge tube.

(II) Centrifuge at 15,000g for 10s to pellet the cells, remove the supernatant.

(III) Add 1ml of PBS or TE buffer to the pellet, vortex or pipet to completely resuspend the pellet.

Optional: If RNA-free DNA is desired, add 20 µl RNaseA solution and incubate at RT for 5 min.

(IV) Add 100 µl of **Proteinase K Solution** and 1ml **Binding Buffer**(vortex before use)**,** vortex thoroughly to lyse the cells and proceed **directly** to **Step 6.**

b. Animal tissue (＜100 mg, < 80 mg for spleen) or insect (< 100 mg):

***Do not use too much sample, which will clog the column and obtain lower yield and quality.**

Three methods can be used for tissue treatment:

(I) Cut the tissue into small pieces, add 1 ml of **Lysis Buffer,** Proceed to **Step 2**.

(II) Add 1 ml of **Lysis Buffer** to tissue and homogenize for 10s using a small homogenizer, then transfer to 50ml centrifuge tube. Proceed to **Step 2**.

(III) Tissue may be ground in liquid nitrogen using a mortar and pestle. After grinding, transfer the ground tissue to 50ml centrifuge tube, then add 1 ml of **Lysis Buffer** and proceed to **Step 2**.

2. Add 100 µl of **proteinase K stock solution**, mix by vortexing.

** Do not premix Lysis Solutionr and proteinase K solution before use, proteinase K may undergo self-digestion without substrate.*

3. Incubate at 56℃ in water-bath or incubator for 1- 3 h or longer **until completely lysed.**, pulse vortex 5-10 sec occasionally during incubation.
4. **Optional:** If RNA-free DNA is desired, add 20 µl RNaseA solution and incubate at RT for 5 min.
5. Add 1 ml of **Binding Buffer**(vortex before use) into reaction, mix well by vortex.
6. **Incubating at 70°C in water-bath or heating block for 15 min.**

   ** A white precipitate may form after addition of Binding Buffer, which will redissolve during incubation at 70°C, and will not affect the DNA binding.*

   ** Some tissue debris (i.e. zebra fish bone or insect exoskeleton) may not be digestible, it is important to remove the debris by centrifuging at (10-15,000 ×g) for 5 min before loading on column, since these debris will clot the column.*
7. Add 1 ml **Ethanol**, mix thoroughly by vortex and apply the solution to midi spin column with collection tube, spin at top speed (10-15,000 ×g) for 5 min.

   ** A precipitate may form after addition of Ethanol, **apply all the solution and precipitate** to the column.*
8. Discard the flow-through, wash **twice** with 3 ml of **Wash Buffer** by spin at 15,000 ×g for 5 min.
9. Discard the flow-through, wash with 1ml of **Wash Buffer II** by centrifuge at 10,000×g for 5 min. to remove ethanol.
10. Discard the flow-through, Elution twice with 250-500uL **Elution Solution** or **H_2O** (pH must between 7.0-8.5), Incubate at 56℃ for 5 min, to elute the DNA by centrifugation for 5 min, and store the DNA at -20℃.

    * When using water to elute, make sure the pH value is within 7.0-8.5. Lower pH may cause lower DNA recovery.