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Tissue & Cell Genomic DNA Purification Kit

Cat. #：**DP021/ DP021-150**

Size：**50/150** reactions

Store at RT

For research use only

Description:

The **Tissue & Cell Genomic DNA Purification Kit** provides a rapid, simple, and effective approach to isolate the genomic DNA from various animal tissues, culture cells and bacteria. This kit does not require organic solvents such as phenol and chloroform, it is safe and user-friendly. The isolation process is based on a spin column format, which involves cell lysis by proteinase K, adsorption of DNA to the column, washing and elution of DNA from the column. The Tissue & Cell Genomic DNA Purification Kit is designed for isolating DNA from 1~25 mg tissue samples (< 10 mg spleen sample), up to 5 x 10^6 cultured tissue cells, or < 2 x 10^9 bacterial cells. Typical yield ranges from 2~40 µg of DNA, depending upon the sample volume and sample type. The purified DNA is suitable for various applications, including PCR, restriction enzyme digestion, cloning, and dot blot analysis.

Components of the Kit:

	DP021	DP021-150
1. Extraction Solution*	12 ml	36 ml
2. Binding Solution	30 ml	90 ml
3. Proteinase K Powder**	22 mg	22 mg x 3
4. RNase A Powder**	44 mg	44 mg x 3
5. Wash Solution	16 ml **(add 64 ml of Ethanol before use)**	48 ml **(add 192 ml of Ethanol before use)**
6. Elution Solution	20 ml	60 ml
7. Spin Column	50 pcs	150 pcs
8. Collection Tube	50 pcs	150 pcs

* **Upon storage at low temperature, Extraction Solution may form SDS precipitate, which can be easily dissolved by incubating the bottle at 35°C.**

** **Store the lyophilized Proteinase K & RNase A at -20°C, store all other reagents and kit components at room temperature(25~28°C).**

** **Proteinase K may turn yellow after long term storage. This is a normal phenomenon.**

Equipment and reagents to be supplied by the user:

- For tissue grinding: Small homogenizer (fisher Tissue Tearor. Polytron or Turax). Alternatively, mortar and pestle.
- Trypsin (for adherent tissue culture cells only)
- PBS buffer or TE buffer (culture cells only)
- 180ul Lysozyme (for gram-positive bacteria strains) [8 mg lysozyme powder in 20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 1.2% Triton X-100].
- 100% Ethanol

Things to do before starting :

- Dissolve Proteinase K Powder in 1.1 ml sterile H_2O and store at -20°C.
- If RNA-free genomic DNA is desired, prepare RNase A solution by dissolving RNase A powder in 220 µl sterile H_2O and store at -20°C.
- Preheat water bath or heat block at 37°C ,56°C, 70°C,60~65°C respectively.

General Procedures:

*** Please follow suggested sample amount to avoid clogging of column, reducing DNA quality and yield.**

1. **A. Cultured cells from tissue** (1- 5 X 10^6):
   (1) Harvest the cells (for adherent cells, trypsinize cells before harvesting) and transfer to 1.5 ml microcentrifuge tube.
   (2) Centrifuge at 14,000 x *g* for 10 seconds to pellet the cells, carefully remove the supernatant.
   (3) Add 200 µl of PBS or TE buffer to the pellet and completely resuspend the pellet by vortexing or pipetting.
   (4) Add 200 µl of **Extraction Solution** to lysis. Proceed to **Step 2**.

   B. Animal or insect tissue (< 25 mg), **for spleen** (< 10 mg)**:**

   There are three methods available for tissue treatment:

(1) Cut the tissue into small pieces and add 200 µl of **Extraction Solution,** Proceed to **Step 2**.

(2) Add 200 µl of **Extraction Solution** to tissue and homogenize for 10 seconds using a small homogenizer, then transfer to 1.5 ml microcentrifuge tube. Proceed to **Step 2**.

(3) Homogenize the tissue in liquid nitrogen and transfer the homogenate to 1.5 ml centrifuge tube. Add 200 µl of **Extraction Solution** and proceed to **Step 2**.

C. Bacteria:

(1) Gram-negative bacteria: Centrifuge cells (< 2 x 10^9) at top speed (12~14,000x *g*) for 2 min, carefully remove the supernatant and resuspend bacterial pellet in 200 µl **Extraction Solution.** Mix by vortexing or pipetting. Proceed to **Step 2**.

(2) Gram-positive bacteria: Centrifuge cells (< 2 x 10^9) at top speed for 2 min, and carefully remove the supernatant.
Incubate the pellet in -80°C for 5 min (or in liquid nitrogen for 30 seconds), and then immediately incubate the tube in 37°C for 2 min. Resuspend the pellet in 180 µl of **Lysozyme Solution**. Mix by vortexing or pipetting. Incubate at 37°C for 30 min and add 200 µl **Extraction Solution.** Proceed to **Step 2**.

D. Yeast cell:

Please refer to appendix on page 6.

2. Add 20 µl of **Proteinase K Solution** to the microcentrifuge tube, mix by vortexing.
** Do not premix Extraction Buffer and proteinase K solution before use to prevent proteinase K from undergoing self-digestion without substrate.*

3. Incubate samples at 56°C in water bath or incubator for 0.5~3 hours or longer **until complete lysis of tissue (time varies depending on sample size)**, vortex 5~10 seconds at frequent intervals during incubation.

Please refer to the table as below.

Sample	Time
Cultured cells from tissue (< 5 X 10^6)	0.5-1 hours
Animal or insect tissue (< 25 mg), for spleen (< 10 mg)	1-3 hours
Bacteria (< 2 x 10^9)	0.5-1 hours
Yeast cell (< 5 x 10^7)	0.5-1 hours

4. To remove tissue debris, centrifuge at 14,000 x *g* for 5 min after lysis is complete. Transfer the clear lysate to a new 1.5 ml microcentrifuge tube.

5. **Optional:** If high quality DNA (no RNA contamination) is desired, add 4 µl RNaseA solution and incubate at RT for 5 min.

6. Add 200 µl of **Binding Solution** to the lysate, mix by vortexing.

7. Incubating at 70˚C water bath or heating block for 10 min.

** A white precipitate may form after addition of Binding Solution, which dissolves during incubation at 70˚C. For some tissues, the mixture may not turn clear (e.g. brain tissue), however, this does not affect DNA binding. The mixture will turn clear after addition of ethanol.*

8. Add 200 µl **Ethanol**, mix thoroughly by vortexing, and transfer the mix (including any precipitates) to spin column mounted into a collection tube. Centrifuge at top speed for 1 min.

** A precipitate may form after addition of ethanol,* ***please do not spin. Transfer the mix (including the solution and precipitate)*** *to the column directly.*

9. Discard the filtrate, add 300 µl **of Binding Solution** to the column and centrifuge at top speed for 1 min.

** This step is essential for removal of endonuclease to prevent Contamination.*

10. Discard the filtrate, add 700 µl of **Wash Solution** and centrifuge at top speed for 1 min. **Repeat this step once more**.

11. Discard the filtrate and centrifuge for additional 5 min at top speed to remove residual trace of ethanol.

** If centrifugation speed is lower than 12,000x g or residual ethanol must be removed completely, incubate the spin column in a heat oven (60~65°C) for 5 min to evaporate all of the ethanol.*

12. Transfer the **spin column** into a new microcentrifuge tube and add 100~200 µl preheated (60~65°C) **Elution Solution** or **H_2O (pH 7.0~8.5)** into the column and wait for 1~2 min.

13. Centrifuge at top speed for 1 min to elute the DNA. Store the eluted DNA at -20°C.

** Repeating elution once will increase DNA yield by 10~15%, though DNA will be diluted.*

Appendix: protocol for isolation of genomic DNA from yeast

Additional reagents required:

- Sorbitol buffer **(fresh prepared)**: 1 M sorbitol
  100 mM EDTA
  14 mM β-mercaptoethanol
- Lyticase (ICN Biomedical) or zymolyase (Zymo Research)
  Ex: yeast lytic enzyme 5g, add water 200μl(final conc.50U/μl),aliquot to 5-10 tubes, add 4μl per reaction.

Procedures:

1. Spin the cells (< 5x 10^7) at 5000 x *g* for 10 min and carefully remove the supernatant.

2. Resuspend the pellet in 600 µl Sorbitol buffer, add about 200 U lyticase or zymolyase and incubate at 30°C for 30~60 min.
** Incubation time may vary depending on cell numbers, species, and enzyme activity. See the guideline from enzyme supplier to monitor the incubation time.*

3. Spin down the spheroplasts at 300 x *g* for 10 min.
**** The cells may be fragile after enzyme digestion, centrifuge with less g force.***

4. Resuspend the spheroplasts in 200 µl **Extraction Solution**.

5. Continue to step 2 as per **General Procedure** on page 3.

Troubleshooting Guide

Comments and Suggestion	
column is clogged	
a) Too much sample	Reduce the sample volume
b) Sample was not lysed completely.	1) Grind the tissue as fine as possible. 2) Mix frequently during digestion to ensure more efficient lysis or extend the incubation time in the **Step 3.**
Poor DNA yield	
a) Wash Solution was not prepared properly.	Make sure to add ethanol to Wash Solution before use.
b) Sample was not lysed completely	1) Grind the tissue as fine as possible. 2) Do not premix the solutions while performing the procedures.(e.g., Do not mix proteinase K and Extraction solution.) 3) Mix frequently during digestion to ensure more efficient lysis or extend the incubation time in the **Step3.**
c) DNA was not eluted properly.	1) DNA should be eluted only in low-salt Solution [e.g., 10 mM Tris-HCl, pH 8.5 (Elution solution) or water]. Elution efficiency is dependent on pH. The maximum efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH is within this range. 2) Ensure that Elution Solution is added at the center of the membrane and is completely absorbed. 3) Allow Elution Solution to incubate in the Binding plate for longer time or elute twice to increase the DNA

	recovery.
d) DNA is sheared or degraded	1) Maintain a sterile environment while working to avoid DNase contamination. 2) Avoid repeated freezing and thawing of DNA samples.

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