Tissue Genomic DNA Extraction MicroElute Kit DE-076

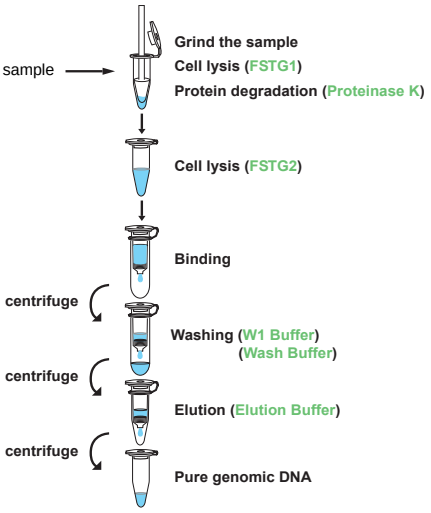
The Tissue Genomic DNA Extraction MicroElute Kit simplifies purification of genomic DNA with a fast spin-column procedure. Optimized protocols are included for use with small amounts of fresh , frozen tissues , (paraffin formalin) fixed tissues, bacteria (culture, biological fluids, swabs) yeast and dried blood spots,.

For purification of genomic DNA from small tissue samples

Features and Specifications

◊ **Simple, Pure and Fast** :
Quick and easy to use
Versatile : Extract gDNA from 25mg of paraffin-embedded tissues, formalin-fixed tisues, biological fluids, bacteria (including Gram+) yeast or dried blood
No phenol-chloroform extraction or ethanol precipitation required

◊ **High recovery** :
Purify genomic DNA : 10 ul elution volume
Maximal column binding capacity :15 ug DNA



Kit components	DE-076 50 PREPS	DE-077 100 PREPS	DE-078 300 PREPS
FSTG1 Buffer	15 ml	30 ml	70 ml
FSTG2 Buffer	15 ml	30 ml	70 ml
W1 Buffer*	22 ml	44 ml	124 ml
Wash Buffer**	10 ml	20 ml	50 ml
Elution Buffer	15 ml	30 ml	90 ml
Proteinase K	11 mg	11 mg x 2	11 mg x 6
TGM Column	50 pcs	100 pcs	300 pcs
Elution Tube	50 pcs	100 pcs	300 pcs
Micropestle	50 pcs	100 pcs	300 pcs
Collection Tube	100 pcs	200 pcs	600 pcs

***Add 8 / 16 / 45 ml ethanol (96-100%) to W1 Buffer when first open.**

****Add 40 / 80 / 200 ml ethanol (96-100%) to Wash Buffer when first open.**

Important Notes:
1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add 1.1 ml sterile ddH2O to Proteinase K tube to make a 10 mg/ml stock solution. Vortex and make sure that Proteinase K has been completely dissolved. Store the stock solution at 4 °C.
3. Add 8 / 16 / 45 ml ethanol (96- 100 %) to W1 Buffer when first open.
4. Add 40 / 80 / 200 ml ethanol (96- 100 %) to Wash Buffer when first open.
5. Prepare two dry baths or two water baths before the operation:one to 60 °C for step 4 and the other to 70 °C for step 7.
6. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.

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General Protocol:

Please Read Important Notes Before Starting The Following steps.
For other special samples, please refer to Special Protocol and choose the appropriate one.

(For fresh sample)

1.Cut up to 25 mg tissue sample to a microcentrifuge tube (not provided). Use provided Micropestle to grind the tissue sample a few times. Or you can grind the tissue sample in liquid nitrogen then transfer the powder to a new microcentrifuge tube. (not provided)

(For frozen sample)

1. Weigh up to 25 mg tissue sample and grind the tissue sample in liquid nitrogen then transfer the powder to a new microcentrifuge tube. (not provided)---If DNA is prepared from spleen tissue, no more than 10 mg should be used.

2. Add 200 µl F TG1 Buffer and mix well by micropestle or pipette tip.
3. Add 20 µl Proteinase K (10mg/ml) to the sample mixture. Mix thoroughly by vortexing.
4. Incubate at 60 °C until the tissue is lysed completely. Vortex every 10~15 min during incubation to break up the tissue sample.
5. Centrifuge the tube at 4,500 x g for 1 minute to pellet the debris and transfer the clarified lysate to a new microcentrifuge tube.
6. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate for 2 min at room temperature.
7. Add 200 µl F TG2 Buffer to the sample mixture, mix thoroughly by pulse-vortexing and incubate at 70 °C for 10 min.
8. Briefly spin the tube to remove drops from the inside of the lid.
9. Add 200 µl ethanol (96-100%) to the sample. Mix thoroughly by pulse-vortexing.
10. Briefly spin the tube to remove drops from the inside of the lid.
11. Place a TGM Column in a Collection Tube. Transfer the mixture (including any precipitate) carefully to TGM Column. Centrifuge for 1 min then place TGM Column to a new Collection Tube.
12. Wash TGM Column with 500 µl W1 Buffer by centrifuge for 1 min then discard the flow-through.
13. Wash TGM Column with 750 µl Wash Buffer by centrifuge for 1 min then discard the flow-through. ---Make sure that ethanol has been added into Wash Buffer when first open.
14. Centrifuge for an additional 3 min to dry the column.
    Important Step! The residual liquid can affect the quality of DNA and inhibit subsequent enzymatic reactions.
15. Place TGM Column to Elution Tube.
16. Add 10 µl of Elution Buffer or ddH2O (pH 7.5-9.0) to the membrane center of TGM Column.
    Stand TGM Column for 3 min.
    Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
17. Centrifuge for 2 min to elute total DNA.
18. Store total DNA at 4°C or -20°C.

For Fixed Tissues

For paraffin-embedded tissues

1. Cut up to 25 mg paraffin-embedded tissue sample to a microcentrifuge tube (not provided).
2. Add 1 ml xylene, mix well and incubate at room temperature for 30 min.
3. Centrifuge at full speed for 5 min. Remove the supernatant by pipetting.
4. Add 1 ml ethanol (96- 100 %) to the deparaffined tissue, mix gently by vortexing.
5. Centrifuge at full speed for 5 min. Remove the supernatant by pipetting.
6. Incubate at 37 °C for 10 min to evaporate ethanol residue.
7. Grind the tissue sample by micropestle or liquid nitrogen and follow the General Protocol starting from step 2.

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For formalin-fixed tissues

1. Wash 25 mg tissue sample twice with 1 ml PBS to remove formalin.
2. Grind the tissue sample by micropestle or liquid nitrogen and follow the General Protocol starting from step 2.

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Special Protocol (For Bacterial Culture)

I-For bacterial cultures

1. Transfer 1 ml well-grown bacterial culture to a microcentrifuge tube (not provided).
2. Descend the bacterial cells by centrifuging at full speed for 2 min and discard the supernatant completely.
3. Follow the General Protocol starting from step 2.

II. For bacterial in biological fluids

1. Collect bacteria by centrifuging biological fluids at 7,500 rpm (5,000 x g) for 10 min.
2. Follow the General Protocol starting from step 2.

III. For bacteria from eye, nasal, pharyngeal, or other swabs

1. Soak the swabs in 2 ml PBS at room temperature for 2- 3 hr.
2. Collect bacteria by centrifuging at 7,500 rpm (5,000 x g) for 10 min.
3. Follow the General Protocol starting from step 2.

IV.For Gram-positive bacterial

HINT:
Preheat two dry baths or two water baths before the operation: one to 60 °C and the other to 95 °C for step 7.

1. Transfer 1 ml well-grown bacterial culture to a microcentrifuge tube (provided by user).
2. Descend the bacterial cells by centrifuging at full speed for 2 min and discard the supernatant completely.
3. Resuspend the cell pellet in 200 µl lysozyme reaction solution (20 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2mM DTA; 1.2 % Triton).
4. Incubate at 37 °C for 30 min.
5. (Optional): If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate for 2 min at room emperature.
6. Add 20 µl Proteinase K to the sample, and then add 200 µl F TG2 Buffer to the sample. Mix thoroughly by pulse-vortexing.
7. Incubate at 60 °C for 30 min and then for a further 15 min at 95 °C.
8. Follow the General Protocol starting from step 8.

For Yeast

1. Transfer 3 ml log-phase (OD600 = 10) yeast culture to a microcentrifuge tube (not provided).
2. Descend the yeast cells by centrifuging at 7,500 rpm (5,000 x g) for 10 min and discard the supernatant completely.
3. Resuspend the cell pellet in 600 µl sorbitol buffer (1M sorbitol; 100 mM EDTA; 14 mM ß-mercaptoethanol). Add 200 U zymolase or lyticase at 30 °C for 30 min.
4. Centrifuge at 7,500 rpm (5,000 x g) for 5 min. Remove the supernatant by pipetting.
5. Follow the General Protocol starting from step 2.

For Dried Blood Spot

HINT:
Preheat three dry baths or three water baths before the operation: one to 85 °C for step 2, another to 60 °C for step 5 and the other to 70 °C for General Protocol step 7.

1. Cut the filter paper (e.g. S&S903) with dried blood spot into a microcentrifuge tube.
2. Add 200 µl F TG1 Buffer and incubate at 85 °C for 10 min.
3. Briefly spin the tube to remove drops from the inside of the lid.
4. Add 20 µl Proteinase K to the sample mixture. Mix thoroughly by vortexing.
5. Incubate at 60 °C for 1 hr. Vortex every 10- 15 min during incubation.
6. Follow the General Protocol starting from step 5.

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