YTA-Tissue DNA Extraction mini Kit

Cat No :YT9020 **Size: 50 preps** <<for research use only>>

Kit Contents	50 preps
Lysis buffer	15 ml
Binding Buffer	15 ml
Proteinase K 20mg	1 vial
Proteinase K stock buffer 1 ml	1 vial
W1 Buffer * (concentrate)	22 ml
W2 Buffer ** (concentrate)	10 ml
Elution Buffer	15 ml
Mini Column & collection tube	50 sets
Elution tube	50 PCS

Materials be supplied by the users :
Ethanol (96–100%)

Preparation note:
Add 5.5 ml ethanol (96-100%) to W1
Add 40 ml ethanol (96-100%) to W2
Add 1ml Proteinase K stock buffer to proteinase K Powder , mix by vortex and keep it at -20°C

Protocol: Isolation of DNA from Animal Tissue
Hint: Set dry or water baths: 60°C for step 4 and 70°C for step 6.
1. Cut tissue sample (up to 25 mg) to a microcentrifuge tube (not provided). Use provided Micropestle to grind the tissue sample. Or you can grind the tissue sample in liquid nitrogen with mortar and pestle then transfer the powder to a microcentrifuge tube.
-If DNA is prepared from spleen tissue, no more than 10 mg should be used.
2. Add 200 µl Lysis Buffer and mix well by Micropestle or pipette tip.
3. Add 20 µl Proteinase K to the sample mixture. Mix thoroughly by vortexing.
4. Incubate at 60°C until the tissue is lysed completely (1~3 hrs). Vortex occasionally during incubation.
-Sample can be incubated overnight as well for complete Lysis.
5. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A (not provided). Mix thoroughly by vortexing and incubate at room temperature for 2 mins.
6. Add 200 µl Binding Buffer to the sample mixture, mix thoroughly by pulse-vortexing and incubate at 70°C for 10 mins.
7. Add 200 µl ethanol (96~100%) to the sample mixture. Mix thoroughly by pulse-vortexing.
8. Briefly spin the tube to remove drops which inside of the lid.
9. Place a Mini Column in a Collection Tube. Transfer the mixture (including any precipitate) carefully to the Mini Column. Centrifuge at full speed (~18,000 x g) for 1 min then discard flow-through.
10. Add 400 µl W1 Buffer to the Mini Column. Centrifuge at full speed for 1 min then discard flow-through.
-Make sure that ethanol has been added into W1 Buffer at the first open.
11. Add 750 µl W2 Buffer to the Mini Column. Centrifuge at full speed for 1 min then discard flow-through.
-Make sure that ethanol has been added into W2 Buffer at the first open.
12. Centrifuge at full speed for an additional 3 mins to dry the column.(-Important Step! This step will remove the residual liquid).
13. Add 100 µl of preheated Elution Buffer or ddH2 O (pH 7.5-9.0) to the membrane of the Mini Column. Stand the Mini Column for 3 mins.
-Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
-If less sample to be used, reduce the elution volume to 50 µl to increase DNA concentration and do not elute the DNA using less than suggested volume (50 µl). It will lower the final yield.
14. Centrifuge at full speed for 2 mins to elute DNA.

Protocol: Isolation of DNA from Animal Cultured Cells
Additional requirement: RNase A (optional), 96~100% ethanol, trypsin or cell scraper (for monolayer cell), PBS.
Hint: Set dry or water baths: 60°C and 70°C.
1. Harvest cells
a. Cells grown in suspension
i. Transfer the appropriate number of cells (up to 1×107) to a microcentrifuge tube.
ii. Centrifuge at 300 x g for 5 mins. Discard supernatant carefully and completely.
b. Cells grown in monolayer
i. Detach cells from the dish or flask by trypsinization or using a cell scraper. Transfer the appropriate number of cell (up to 1×107) to a microcentrifuge tube.
ii. Centrifuge at 300 x g for 5 mins. Discard supernatant carefully and completely.
2. Resuspend cell pellet in PBS to a final volume of 200 µl.
3. Follow the Animal Tissue Protocol starting from step 2.

Protocol: Isolation of Genomic DNA and Viral DNA from Blood
Additional requirement: RNase A (optional), 96~100% ethanol, PBS.
Hint: Set dry or water baths: 60°C for step 3 and 70°C for step 4.
1. Transfer up to 200 µl sample (whole blood, serum, plasma, body fluids, buffy coat) to a microcentrifuge tube.
-If the sample volume is less than 200 µl, add the appropriate volume of PBS.
2. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A (not provided). Mix thoroughly by vortexing and incubate at room temperature for 2 mins.
3. Add 20 µl Proteinase K to the sample, and then add 200 µl Binding Buffer to the sample. Mix thoroughly by pulse-vortexing. Incubate at 60°C for 30 mins. Vortex occasionally during incubation.
4. Incubate at 70°C for 10 mins.
5. Follow the Animal Tissue Protocol starting from step 7.

Protocol: Isolation DNA from Bacteria
Additional requirement: • RNase A (optional), 96~100% ethanol.
• For Gram-positive bacteria: lysozyme reaction solution (20 mg/ml lysozyme; 20 mM Tris-HCI, pH 8.0; 2 mM EDTA; 1.2% Triton) is suggested.
Hint: Set dry or water baths: one to 60°C, the other to 70°C.
l. For bacterial cultures
1. Transfer 1 ml well-grown bacterial culture to a microcentrifuge tube (not provided).
2. Descend the cells by centrifuging at full speed for 2 mins and discard supernatant completely.
3. Follow the Animal Tissue Protocol starting from step 2.
ll. For bacterial in biological fluids
1. Collect cells by centrifuging biological fluids at 7,500 rpm (5,000 x g) for 10 mins and discard supernatant completely.
2. Follow the Animal Tissue Protocol starting from step 2.
lll. For bacteria from eye, nasal, pharyngeal, or other swabs
1. Soak the swabs in 2 ml PBS at room temperature for 2~3 hrs.
2. Collect cells by centrifuging at 7,500 rpm (5,000 x g) for 10 mins and discard supernatant completely.
3. Follow the Animal Tissue Protocol starting from step 2.
lV. For Gram-positive bacteria
Hint: Set dry or water baths: one to 37°C, another to 60°C and the other to 95°C.
1. Transfer 1 ml well-grown bacterial culture to a microcentrifuge tube (not provided).
2. Descend the cells by centrifuging at full speed for 2 mins and discard supernatant completely.
3. Resuspend the cell pellet in 200 µl lysozyme reaction solution (20 mg/ml lysozyme; 20 mM Tris-HCI, pH 8.0; 2 mM EDTA; 1.2% Triton). Incubate at 37°C for 30~60 mins.
4. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A (not provided). Mix thoroughly by vortexing and incubate at room temperature for 2 mins.
5. Add 20 µl Proteinase K to the sample, and then add 200 µl Binding Buffer to the sample. Mix thoroughly by pulse-vortexing. Incubate at 60°C for 30 mins and vortex occasionally during incubation.
6. Do a further incubation at 95°C for 15 mins.
7. Follow the Animal Tissue Protocol starting from step 7.