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یکتا تجهیز آزما

Parrafin-fixed Tissue DNA Extraction micro Kit

Cat No : YT9035 Size : 50 preps << For research use only>>

Cat No : YT9036 Size : 4 preps

Components:	**4 preps**	**50 preps**
TG1 buffer	1.5 ml	15 ml
TG2 buffer	1.5	15 ml
Proteinase K(lyophilized)	1mg	11 mg
W1 buffer (concentrate)	1.3 ml	22 ml
Wash buffer (concentrate)	1 ml	10 ml
Elution buffer	1 ml	15 ml
TG micro column	4 PCS	50 PCS
Collection tube	8 PCS	100 PCS
Elution tube	4 PCS	50 PCS

Preparation of Proteinase K solution (10 mg/ml) by adding ddH2O		
ddH2O volume for Proteinase K	0.1 ml	1.1 ml

Preparation of W 1 Buffer and Wash Buffer by adding ethanol (96 ~ 100%)		
Ethanol volume for W1 buffer	0.5 ml	8 ml
Ethanol volume for wash buffer	4 ml	40 ml

Specification :

Principle: mini spin column (silica matrix)
Minimum elution volume: 12 µl
Sample size: < 25 mg fixed tissue

Important Notes:

1. **Additional requirement :** Xylene, RNase A (optional), 96~100% ethanol
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. 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.**
4. Add ethanol (96- 100 %) to **W1 Buffer** and **Wash Buffer** when first open.
5. Prepare dry baths or water baths before the operation: one to 60 °C for step 10 and the other to 90 °C for step 12.
6. Preheat the Elution Buffer to 65 °C for step 19.
7. All centrifuge steps are done at full speed (~ 18,000 x g) in a microcentrifuge.

Protocol: Isolation of DNA from paraffin-fixed tissue

Please Read Important Notes Before Starting Following Steps.

1. Add up to 25 mg of paraffin slice sample to a microcentrifuge tube.

2. Add 1 ml xylene and mix well. Close the lid and vortex vigorously for 10 sec. Incubate the sample at room temperature until
the paraffin is dissolved completely.
3. Centrifuge at full speed for 5 min. Remove the supernatant by pipetting.
4. Add 1 ml ethanol (96- 100 %) to the deparaffined tissue and mix gently by vortexing.
5. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.
6. Repeat step 4 and 5.
7. Incubate at 37 °C for 10 ~15 min to evaporate ethanol residue completely.
8. Add 200 µl TG1 Buffer and mix well.
9. Add 20 µl Proteinase K (10mg/ml) to the sample mixture. Mix thoroughly by vortexing.
10. **Incubate at 60 °C until the tissue is lysed completely (1~3 h).** Vrotex occasionally during incubation.
--- Sample can be incubated overnight as well for complete lysis.
11. **(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 min.
12. **Incubate at 90 °C for 30 min.** Vrotex occasionally during incubation.
13. Add 200 µl TG2 Buffer to the sample mixture, mix thoroughly by pulse-vortexing.
14. Add 200 µl ethanol (96-100%) to the sample mixture. Mix thoroughly by pulse-vortexing.
15. Place a TG Micro Column in a Collection Tube. Transfer the mixture carefully to the TG Micro Column. Centrifuge at full
speed (~18,000 x g) for 1 min **then place the TG Micro Column to a new Collection Tube.**
16. Add 400 µl W1 Buffer to the TG Micro Column. Centrifuge at full speed for 1 min then discard flow-through.
---Make sure that ethanol has been added into W1 Buffer when first open.
17. Add 650 µl Wash Buffer to the TG Micro Column. Centrifuge at full speed for 1 min then discard flow-through.
---Make sure that ethanol has been added into Wash Buffer when first open.
18. Centrifuge at full speed for an additional 3 min to dry the column.
--- Important Step! This step will remove the residual liquid.
19. Add 12 µl of preheated Elution Buffer or ddH2O (pH 7.5-9.0) to the membrane of the TG Micro Column. Stand the TG Micro
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.
20. Centrifuge at full speed for 2 min to elute DNA.