TIANGEN®

RNAprep Pure FFPE Kit

For purification of total RNA from formalin-fixed, paraffin-embedded tissue sections

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For Research Use Only. Not for use in diagnostic procedures.

RNAprep Pure FFPE Kit

(Spin Column)

Cat. no. GDP439

Kit Contents

	Contents	GDP439 (50 preps)
GDP439H	Buffer RF	12 ml
	Buffer RB	12 ml
	Buffer RW1	40 ml
	Buffer RW	12 ml
	Proteinase K	500 μl
	RNase-Free ddH_2O	40 ml
	RNase-Free Columns CR3 set	50
	RNase-Free Centrifuge Tubes (1.5ml)	50
GRT411	RNase-Free DNase I (1500 U)	1
	Buffer RDD (DNA Digest Buffer)	4 ml
	RNase-Free ddH_2O	1 ml

Note: GDP439H, GRT411 are shipped and packaged separatly.

Storage

Store RNase-Free DNase I and Buffer RDD at 2-8°C; all other reagents can be stored at room temperature. Buffer RF with β-mercaptoethanol added can be stored at 2-8°C for one month.

Introduction

RNAprep Pure FFPE Kit is specially designed for purification of total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. Due to fixation and embedding conditions, nucleic acids in FFPE samples are usually heavily fragmented and chemically modified by formaldehyde. Therefore, nucleic acids isolated from FFPE samples is often of a lower molecular weight than those obtained from fresh or frozen samples. RNA obtained by this kit could be used for downstream experiment as RT-PCR.

Notes <u>Please read these notes carefully before using this kit.</u>

1. Add β-mercaptoethanol to Buffer RF to a final concentration of 1% (e.g., add 10 μl of β-mercaptoethanol to 990 μl of Buffer RF). This lysis buffer is best prepared fresh before use. The prepared Buffer RF can be stored at 2–8°C for up to one month. Note that precipitation may occur during storage of Buffer RF; if any precipitate is observed, heat to dissolve completely before use.
2. Add ethanol (96-100%) to Buffer RW before start as indicated on the bottle.
3. **Preparation of DNase I stock solution**

   Dissolve the lyophilized DNase I (1500 U) in 550 μl of the RNase-Free ddH_2O. Mix gently by inverting. Do not vortex. Divide it into single-use aliquots, and store at -30~-15°C for up to 9 months.

   Note: Thawed aliquots from -30~-15°C could be stored at 2-8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Starting Material

1. Standard formalin-fixation and paraffin-embedding procedures always result in significant fragmentation and crosslinking of nucleic acids. To limit the extent of nucleic acid fragmentation and crosslinking, be sure to:
   - Fixate tissue samples in 4-10% formalin buffer as quickly as possible after surgical removal;
   - Fix for 14-24 hours (longer fixation time leads to over-fixation and more severe nucleic acid fragmentation, resulting in poor performance in downstream assays);
   - Thoroughly dehydrate samples prior to embedding.
2. The starting material for RNA purification should be freshly cut sections of FFPE tissue, each with a thickness of up to 10 μm. Thicker sections may result in lower nucleic acid yields. Up to 8 sections, each with a thickness of up to 10 μm and a surface area of up to 250 mm^2, can be combined in one preparation.

3. If there is no information about the nature of your starting material, we recommend starting with no more than 2 sections per preparation. Depending on RNA yield and purity, it may be possible to use up to 8 sections in subsequent preparations.

Protocol

1. Using a scalpel to cut the sample block into 5-10 µm thick sections.

   Note: If the sample surface has been exposed to air, discard the first 2-3 sections.

2. Immediately place 2-8 sections in a 1.5 ml RNase-Free microcentrifuge tube, add 1 ml xylene, and vortex vigorously for 10 s.
3. Centrifuge for 2 min at 12,000 rpm (~13,400 x g) at room temperature (15-30°C).
4. Carefully aspirate the supernatant with pipette, do not disturb the pellet.
5. Add 1 ml ethanol (96-100%) to pellet, vortex to mix.
6. Centrifuge for 2 min at 12,000 rpm (~13,400 x g) at room temperature (15-30°C).
7. Carefully aspirate the supernatant with pipette, do not disturb the pellet (Use a new pipette tip to aspirate the remained ethanol).
8. Open the tube lid, incubate for 10 min at room temperature or 37°C until all residual ethanol has evaporated.

   Note: It's important to remove residual ethanol, since ethanol carryover may interfere with RNA isolation.

9. Add 200 µl Buffer RF and 10 µl Proteinase K into the pellet, vortex to mix thoroughly.
10. Incubate at 55°C for 15 min, and then at 80°C for 15 min.
11. Centrifuge for 5 min at 12,000 rpm (~13,400 x g) at room temperature, then transfer the supernatant to a new RNase-Free microcentrifuge tube.
12. Add 220 µl Buffer RB and vortex to mix.
13. Add 660 µl ethanol (96-100%) and vortex to mix (precipitate may forms).
14. Transfer 700 µl of solution and precipitate to Spin Column CR3 (put CR3 into collection tube), centrifuge for 1 min at 12,000 rpm (~13,400 x g). Discard the flow-through in the collection tube, and put the spin column back to the collection tube.
15. Repeat step 14, until all of the buffer and precipitate go through the Spin Column CR3, discard the flow-through in the collection tube and put the spin

column back to the collection tube.

16. Preparation of DNase I working solution: transfer 10 µl DNase I stock solution to a new RNase-Free microcentrifuge tube, add 70 µl Buffer RDD, mix gently.
17. Add 80 µl DNase I working solution to the center of Spin Column CR3, incubate for 15 min at room temperature.
18. Add 500 µl Buffer RW1 to the Spin Column CR3, then centrifuge for 30-60 sec at 12,000 rpm (~13,400 x g) at room temperature, discard the flow-through and put the spin column back to the collection tube.
19. Add 500 µl Buffer RW **(Ensure that ethanol (96-100%) has been added)** to the Spin Column CR3, incubate for 2 min at room temperature, then centrifuge for 30-60 sec at 12,000 rpm (~13,400 x g), discard the flow-through and put the spin column back to the collection tube.
20. Repeat step 19.
21. Centrifuge for 2 min at 12,000 rpm (~13,400 x g) at room temperature, discard the flow-through. Open the lid and place the spin column at room temperature for several minutes to dry the membrane completely.

    Note: ethanol carryover into the eluate may interfere with some downstream applications as RT reaction.
22. Place the Spin Column CR3 to a new clean 1.5 ml RNase-Free microcentrifuge tube, and apply 30-100 µl RNase-Free ddH_2O to the center of the membrane. Close the lid and incubate at room temperature for 2 min, then centrifuge at 12,000 rpm (~13,400 x g) for 2 min to obtain RNA buffer.

    Note: The elution buffer should be larger than 30 µl, since small volume will affect recovery ratio. RNA buffer should be stored at -70°C.