TIANGEN

(DP439) RNAprep Pure FFPE Kit

——FFPE

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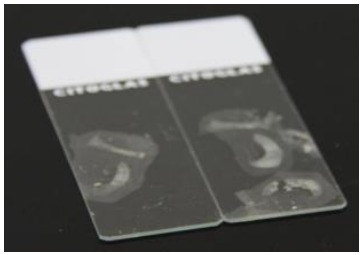
Experiment Preparation

1. Paraffin section or block
2. Ethanol, β-mercaptoethanol; Xylene
3. Disposable sterile syringe (for DNase I preparation); Pipette and matched sterile RNase-Free tips (200 μl ,1 ml)
4. Fume hood; Vortex oscillator; Dry bath; Refrigerated centrifuge



Experiment Preparation - Kit Preparation 1

Please add 96-100% ethanol in Buffer RW before use according to the volume indicated on the label of the bottle, and label the tube.

Preparation of DNase I storage solution

Dissolve the DNase I powder (1500 U) in 550 μl RNase-Free ddH_2O, gently mix well, and store in -20℃ (for up to 9 months) after aliquoting.

Note: The thawed the DNase I storage solution from -20℃ can be stored at 4 ℃ (for up to 6 weeks). Do not freeze again。

Experiment Preparation - Kit Preparation 2

It is suggested to operate this step in the fume hood

Add β-mercaptoethanol in Buffer RF to make 1% final concentration before operating. For example, add 10 μl β-mercaptoethanol to 1 ml Buffer RF. It is suggested to prepare the lysis buffer right before use. The prepared Buffer RF can be placed in 4℃ for one month. Buffer RF may precipitate during storage. If precipitation occurs, please heat and dissolve before use.

Step 1

Cut the paraffin sample into 5-10 μm pieces.

Note: If the surface of the sample is exposed to air, the first 2~3 scraped pieces shall be discarded.

Note: Use fresh FFPE tissue sections less than 10 μm. Too thick sections may result in low RNA yield. The number of sections used for each preparation shall not exceed 8, whose surface area shall not exceed 250 mm^2. If there is no initial sample information, it is recommended that the number of slices used for initial preparation should not exceed 2, and then adjust the number of sections according to the yield and purity of RNA, but not more than 8.

Step 2

Place 2-8 pieces of slides in a 1.5 ml RNase-free centrifuge tube.

Add 1ml xylene, and vortex violently for 10 sec.

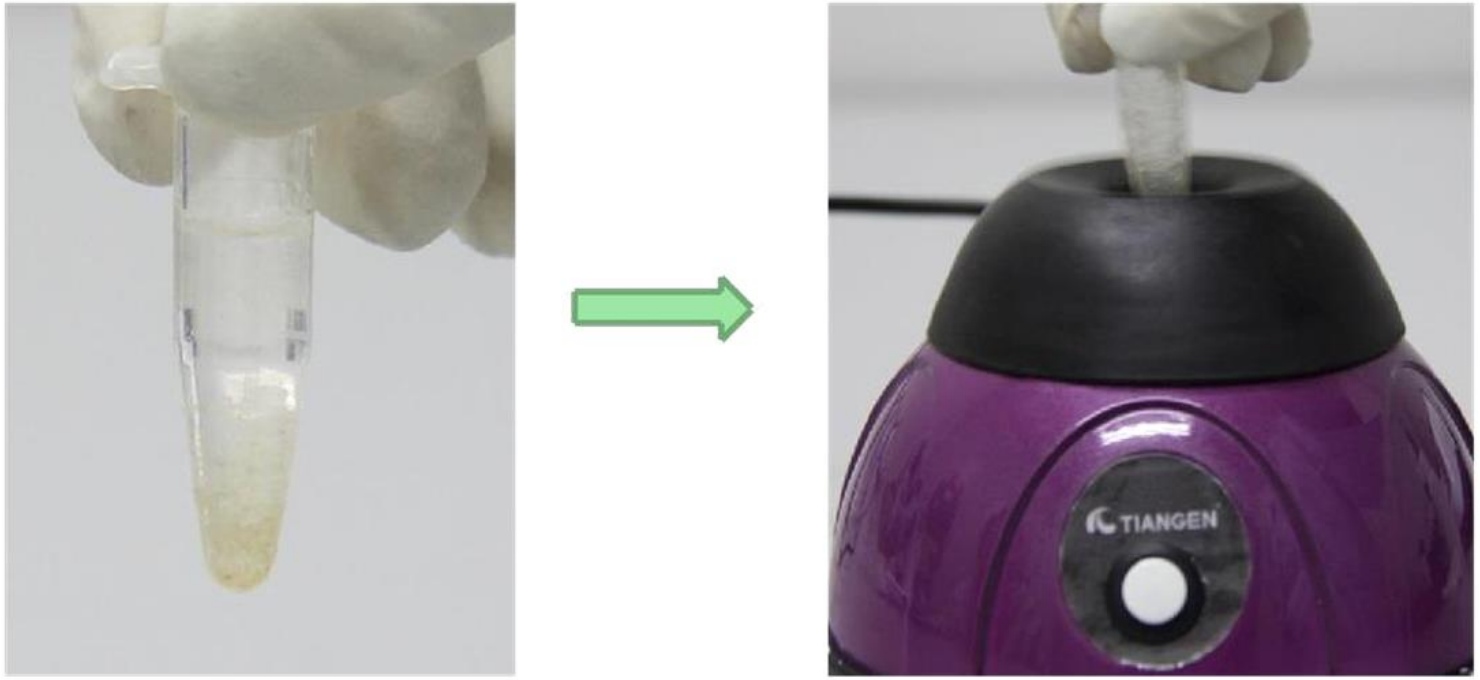
Step 3

Centrifuge at 12,000 rpm (~13,400 g) at room temperature (15-25℃) for 2 min.

Step 4

Carefully remove the supernatant with a pipette without touching the pellet.

Step 5



Add 1ml 96-100% ethanol, and mix thoroughly by vortex.

Step 6

Centrifuge at 12,000 rpm (~13,400 g) at room temperature (15-25℃) for 2 min.

Step 7

Carefully remove the supernatant with a pipette without touching the pellet (remove the residual ethanol with a new tip).

Step 8

Open the tube cap, and place at room temperature for 5-10 min to fully volatilize ethanol.

Note: It is important to completely remove residual ethanol, which can affect RNA.

Step 9

Add 200 μl Buffer RF and 10 μl Proteinase K to the precipitate, and mix well by vortex.

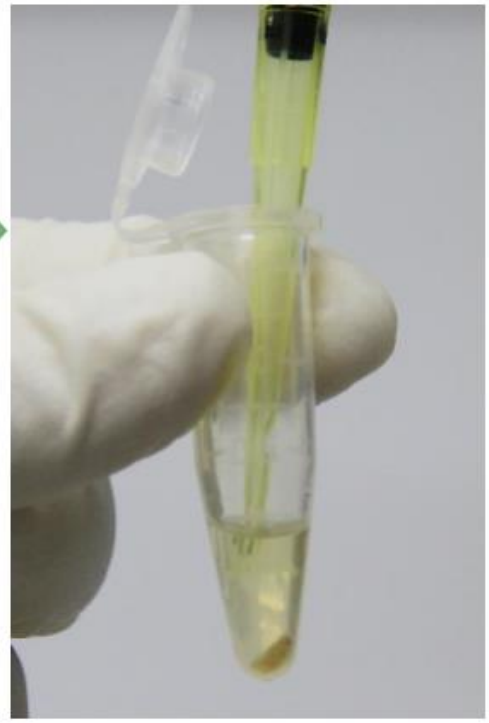
Step 10

Incubate at 55℃ for 15 min.

Then incubate at 80℃ for 15 min.

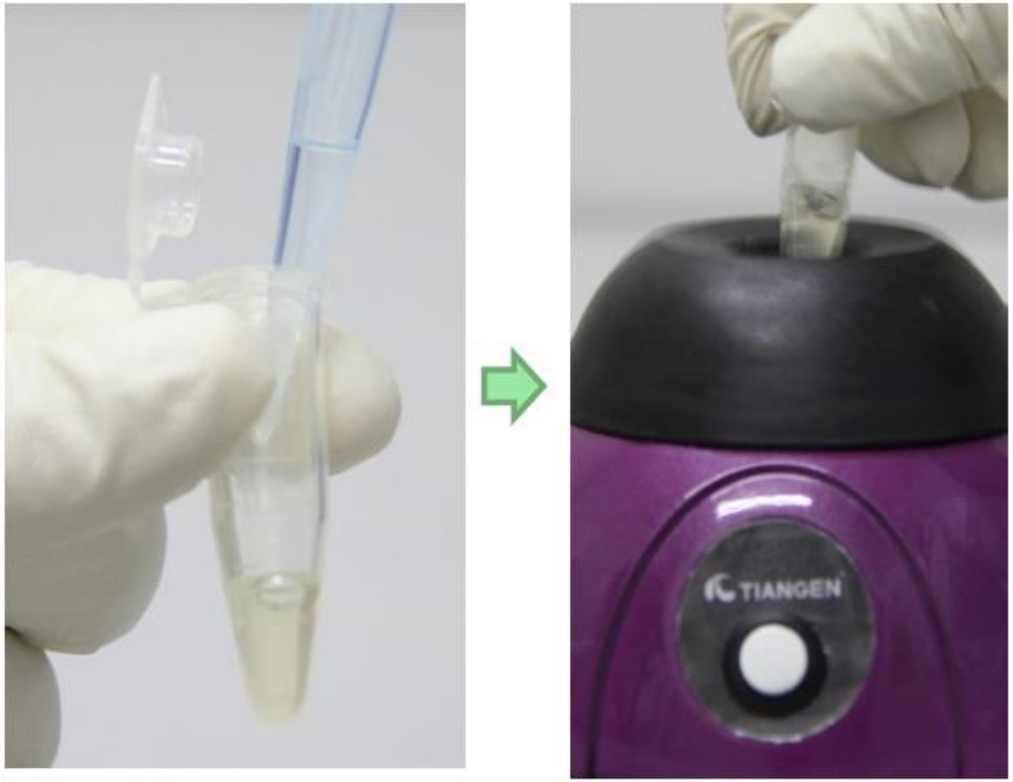
Step 11

Centrifuge at 12,000 rpm (~13,400 g) for 5 min at room temperature (15-25℃).



Transfer the supernatant into a new RNase-free centrifuge tube.

Step 12



Add 220 μl Buffer RB and mix by vortex.

Step 13

Add 660 µl 96-100% ethanol and mix well by vortex (precipitation may occur at this point).

Step 14

Transfer 700 µl solution and precipitate into a Spin Column CR3 (place the Spin Column in the collection tube). Centrifuge at 12,000 rpm (~13,400 g) for 1 min, discard the waste liquid in the collection tube, and place the Spin Column CR3 into the collection tube.

Repeat Step 14 until all the solutions and precipitates pass through the Spin Column CR3 completely. Discard the waste liquid and put the Spin Column CR3 back into the collection tube.

Step 16

Preparation of DNase I working solution:

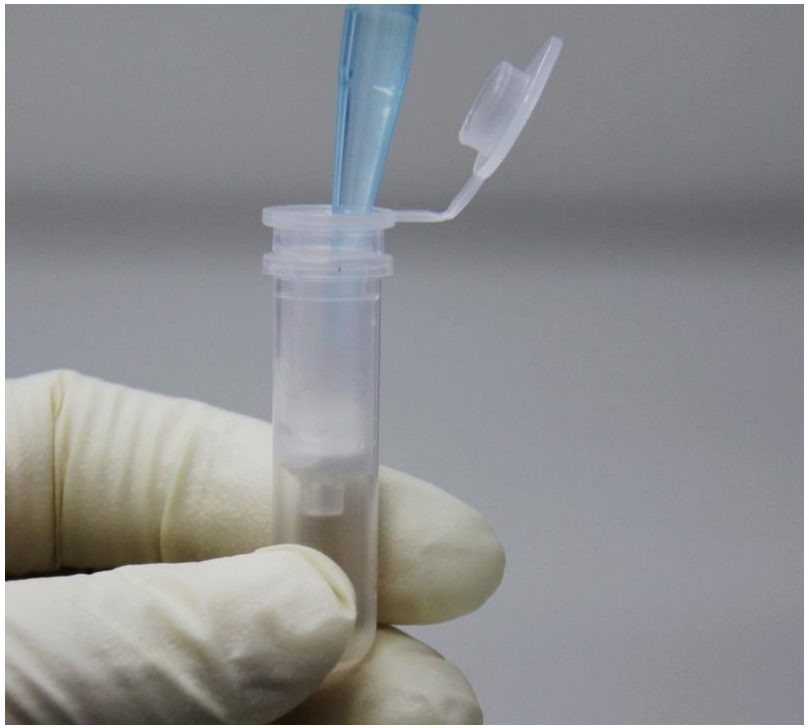
For 1 sample: Add 10 μl of DNase I storage solution into a new RNase-free centrifuge tube, then add 70 μl Buffer RDD and mix well gently (gently mix with a pipette).

For multiple samples extracted at the same time, please prepare the DNase I working solution together. It is recommended to prepare extra solution to avoid the situation that the total amount is insufficient due to the error of the pipette or the liquid attaching to the tips.

Step 17

Add 80 μl DNase I working solution to the center of Spin Column CR3, and place at room temperature for 15 min.

Step 18



Add 500 μl Buffer RW1 in Spin Column CR3. Centrifuge at 12,000 rpm (~13,400 g) for 30-60 sec, discard the waste liquid, and put the Spin Column CR3 back into the collection tube.

Step 19

Add 500 μl Buffer RW to the Spin Column CR3 (**make sure 96-100% ethanol has been added before use**), place at room temperature for 2 min, and centrifuge at 12,000 rpm (~13,400×g) for 30-60 sec. Pour out the waste liquid and put the Spin Column CR3 back into the collection tube.

Step 20 Repeat step 19.

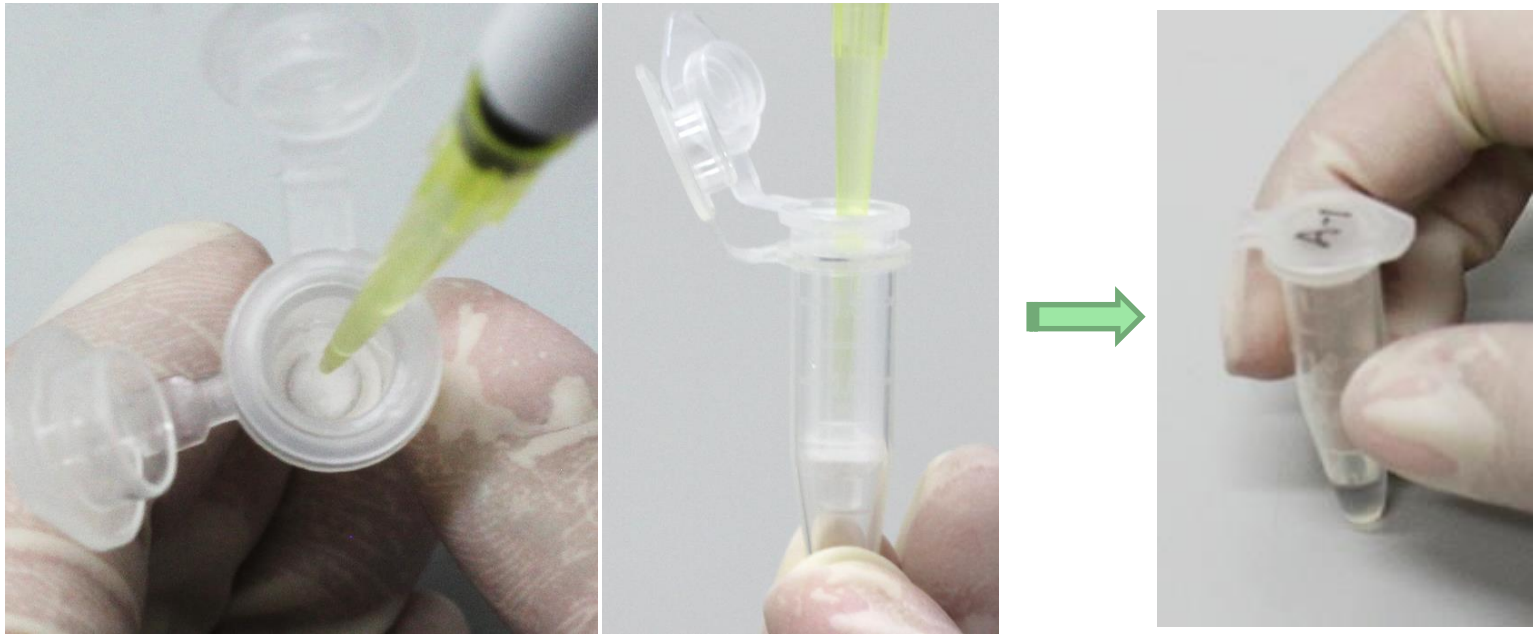
Step 21

Place the Spin Column CR3 in a new 2 ml collection tube. Centrifuge at 12,000 rpm (~13,400×g) for 2 min to remove the residual liquid.

Place the Spin Column CR3 at room temperature for a moment, or place on the clean bench to ventilate for a moment to fully dry the membrane.

Note: Ethanol residues in Buffer RW can inhibit subsequent enzymatic reactions (RT, qPCR, etc.) experiments. However, avoid over-drying, or it will lead to RNA degradation or hard dissolution.

Step 22



Transfer the Spin Column CR3 into the centrifuge tube provided by the kit, add 30–100 µl RNase-Free ddH_2O, place at room temperature for 2 minutes and centrifuge at 12,000 rpm (~13,400×g) for 2 min.

The volume of elution buffer should not be less than 30 µl since too small volume affects the recovery efficiency. RNA should be kept at -70℃ to prevent degradation.