TIANGEN®

miRNAprep Pure FFPE Kit

Highly efficient extraction of RNA>18 nt from paraffin sections

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miRNAprep Pure FFPE Kit

(Spin Column)

Cat.no. GDP502

Kit Contents

	Contents	GDP502 (50 preps)
GDP502H	Buffer RF	12 ml
	Buffer RB	2×12 ml
	Buffer RW	12 ml
	Proteinase K	500 µl
	RNase-Free ddH_2O (bottle)	15 ml
	RNase-Free Columns CR3 set	50
	RNase-Free Centrifuge Tubes 1.5 ml	50
	Handbook	1
GRT411	DNase I (1500 U)	1 tube
	Buffer RDD	4 ml
	RNase-Free ddH_2O (tube)	1 ml

Note: GDP502H, GRT411 are shipped and packaged separately

Storage

DNase I, Buffer RDD and RNase-Free ddH_2O (tube) should be stored at 2-8°C for 15 months. Other reagents should be stored at room temperature (15-30°C) for 15 months.

Introduction

The kit adopts a special buffer system and optimized protocol, which is especially designed for miRNA extraction in formalin-fixed paraffin-embedded tissue sections (hereinafter referred to as FFPE). The kit has the characteristics of high specificity, fast experiment, high quality of extracted miRNA, etc.

RNA extracted by the kit can be applied to downstream tests such as RT-PCR.

Precautions Please read these precautions before use

1. 96%-100% ethanol should be added to Buffer RW before using for the first time. Please refer to the label on the bottle for the added amount.
2. **Preparation of DNase I storage solution**

   Dissolve DNase I dry powder (1500 U) in 550 µl RNase-Free ddH_2O, mix gently, and store at -30~-15°C after aliquoting (can be stored for 9 months).

   Note: DNase I storage solution thawed from -30~-15°C shall be stored at 2-8°C (can be stored for 6 weeks) and shall not be frozen again.

Starting material

1. Standard formalin-fixed paraffin embedding procedures often cause fragmentation of nucleic acids. To minimize the possibility of RNA fragmentation, sample processing should be carried out according to the following operating steps:
   - The tissue should be immersed in 4%-10% formalin solution as soon as possible after excision.
   - The fixing time is preferably 14-24 h (Too long fixing time will lead to more serious RNA fragmentation, which is not conducive to downstream tests);
   - The sample must be completely dehydrated before being coated.
2. Use fresh FFPE tissue slices.The slice thickness should not exceed 10 µm. Too thick slices may cause low RNA yield. The number of slices used for each preparation should not exceed 8 slices, and the surface area should not exceed 250 mm^2.
3. If no information is available on the initial sample, It is suggested that the number of FFPE slices used for initial preparation should not exceed 2 slices, and then the number of slices used for next preparation can be adjusted according to the experimental results, but should not exceed 8 slices.

Protocol

Be sure to add ethanol to Buffer RW as indicated on the bottle and shake thoroughly.

1. Cut the FFPE sample into 5-10 μm thick sheets.

   Note: If the sample surface is exposed to air, discard the first 2-3 pieces.

2. Quickly place 2-8 slices into a 1.5 ml RNase-free centrifuge tube, add 1 ml xylene, and vortex for 10 sec.
3. Centrifuge at room temperature (15-30°C) for 2 min at 12,000 rpm (~13,400×g).
4. Remove the supernatant with the pipette tip and be careful not to disturb the precipitate.
5. Add 1 ml of 96-100% ethanol into the precipitate, and mix well by vortex.
6. Centrifuge at room temperature for 2 min at 12,000 rpm (~13,400×g).
7. Remove the supernatant with a pipette and be careful not to disturb the precipitate. (Use a new pipette tip to carefully aspirate the residual ethanol).
8. Open the tube cover and leave at room temperature or 37°C for 10 min until the residual ethanol volatilizes completely.

   Note: It is very important to completely remove the residual ethanol, or it will affect the extraction of RNA.

9. Add 150 μl of Buffer RF and 10 μl of Proteinase K to the precipitate and thoroughly mixed by vortex.
10. Incubate at 55°C for 15 min and then at 80°C for 15 min.
11. Place on ice for 3 min, centrifuge at 12,000 rpm (~13,400×g) for 15 min, and transfer the supernatant to a new 2 ml RNase-Free centrifuge tube.
12. Add 16 μl of Buffer RDD and 10 μl of DNase I solution, and mix them by turing up and down. Leave at room temperature for 15 min. Centrifuge briefly to collect droplets on the wall and lid of the tube.
13. Add 320 μl of Buffer RB and mix well by vortex.
14. Add 1120 μl of 96%-100% ethanol and mix well by vortex (precipitation may occur).

15. Transfer 700 µl of solution and precipitate into RNase-Free Column CR3 (place the CR3 in the collection tube), centrifuge at 12,000 rpm (~13,400×g) for 1 min, discard the waste liquid in the collection tube, and return the Column CR3 to the collection tube.
16. Repeat step 15 until all the solution and precipitate completely pass through the Column CR3, discard the waste liquid, and put the Column CR3 back into the collection tube.
17. Add 500 µl of Buffer RW **(ensure that ethanol has been added before use)** to Column CR3, let it stand at room temperature for 2 min, centrifuge at 12,000 rpm (~ 13,400 x g) for 30-60 sec, discard the waste liquid, and return Column CR3 to the collection tube.
18. Repeat step 17.
19. Centrifuge at room temperature for 2 min at 12,000 rpm (~13,400×g) and discard the waste liquid. Place the Column CR3 at room temperature for several minutes to completely dry the residual washing buffer in the Column CR3.

    Note: The purpose of this step is to remove the residual Buffer RW in Column CR3. The residual Buffer RW may affect subsequent RT and other experiments.
20. Transfer the Column CR3 into a new RNase-Free centrifuge tube, add 30-100 µl RNase-Free ddH_2O into the middle of the adsorption membrane. Incubate at room temperature for 2 min, then centrifuge for 2 min at 12,000 rpm (~13,400×g).

    Note: The volume of elution buffer should not be less than 30 µl. Too small volume will affect the recovery efficiency. RNA solution should be stored at -70°C.