AFTSpin FFPE DNA Isolation Kit

Cat. No. : RK30112

Product components

Components	Component number	Size 50 RXN
Spin Column 4	RM30183	50 pk
Collection Tube 2mL	RM30192	50 pk
Deparaffinization Buffer	RM30120	40 mL
Digestion Buffer	RM30121	15 mL
Binding Buffer	RM30122	15 mL
Wash Buffer 1	RM30123	13 mL
Wash Buffer 2	RM30124	20 mL
Elution Buffer	RM30125	15 mL
Proteinase K	RM30126	1.8 mL

Note: The above ingredients of different batch/lot numbers should not be used interchangeably; If liquid precipitates, please preheat at 56℃, completely dissolve before use.

Note: Absolute ethanol has been added to Wash Buffer 1 and Wash Buffer 2 in this kit, so it is not necessary to add it by yourself.

Product Description

The kit is based on silicone column purification technology and provides a simple and rapid solution for total DNA extraction from paraffin-embedded tissues and paraffin tissue sections. The extraction process does not require the use of toxic phenol chloroform extraction and time-consuming alcohol precipitation. Tissue samples are directly lysed and digested, and the released nucleic acid are selectively adsorbed on a silicon matrix membrane in an absorption column, and then proteins, inorganic salt ions and many organic impurities are removed in washing step. Finally, the pure nucleic acid is eluted by Elution Buffer.The obtained DNA can be directly used for PCR, Southern Blot, viral DNA detection and other experiments.

Storage

1. This kit is stable for 12 months when stored at room temperature.
2. At low temperature, Digestion Buffer may be precipitated, so it is necessary to completely dissolve the precipitate in a 56℃ water bath.

Scope of Application

It is suitable for total DNA extraction of paraffin-embedded tissues and paraffin tissue sections. The obtained DNA can be directly used for PCR, Southern Blot, viral DNA detection and other experiments.

Precautions

Please read this instruction carefully before the experiment.

1. In order to avoid any potential biological hazard, samples should be considered to have infectious substances and avoid contact with skin and mucous membranes; It is recommended to handle samples in a biosafety cabinet that can prevent the outflow of aerosol. The used test tubes and pipetting tips in the sample preparation area should be discarded into a container containing disinfectant and sterilized before being discarded with other wastes.
2. The components in the kit must be used within expiration date, and not using the components provided by the kit for experiments may lead to abnormal results.
3. Laboratory management should be in strict accordance with the management specifications of PCR gene amplification laboratory, laboratory personnel must be trained professionally, the experimental process should be carried out strictly in different areas (reagent preparation area, sample preparation area, amplification and product analysis area). Used

consumables should be sterilized and then discarded safely.Use of special instruments and equipment should be followed lab rules in each stage of experimental operation. Supplies and consumables are not allowed to cross-use.

4. Use autoclaved disposable centrifuge tubes and tips or use commercial DNA/RNase-free centrifuge tubes and tips.
5. After the nucleic acid extraction of the sample is completed, it is recommended to proceed to the next experiment immediately, otherwise, please store it at -20 ℃ for later use (within 24 hours).
6. At the end of the experiment, the worktable and pipette were treated with 5% hypochlorous acid or 75% alcohol, and then irradiated with ultraviolet lamp for 20 to 30 minutes.

Operational Instructions

Materials to be prepared by the user

1. absolute ethanol
2. xylene (optional, do not need to prepare if non-toxic Deparaffinization Buffer is selected)
3. 1.5 mL RNase-free centrifuge tubes
4. 56℃ and 90℃ water bath

User Protocol

1. Carefully remove excess paraffin with a clean blade, cut the sample (<20 mg) into the smallest pieces or slices, and transfer sample to a 1.5 mL centrifuge tube. For fully digestion, paraffin sample is sliced as small as into 10 - 20 μm. If it is not possible to slice the sample, use scissors or a blade to cut the sample into as small pieces as possible.
2. Remove paraffin according to Plan A or Plan B.

   Plan A: Paraffin removal by xylene

   A1. Add 1 mL xylene to the sample, vortex at high speed for 10 ~ 30 seconds, centrifuge briefly to immerse the sample in xylene, 56℃ water bath for 5 minutes, intense vortex for 15 seconds.

   A2. Centrifuge for 2 minutes at 14,000 x g, carefully discard the supernatant, do not suck precipitate.

   A3. Add 1 mL absolute ethanol, vortex for 10 ~ 30 seconds, centrifuge for 2 minutes at 14,000 x g.

   A4. Thoroughly discard the supernatant, do not pipette up the precipitate.

   A5. Open tube cap and dry at room temperature or 37℃ for 10-15 minutes to thoroughly remove ethanol.

   A6. Proceed to Step 3.

   Plan B:Paraffin removal by non-toxic Deparaffinization Buffer

   B1. Add 0.6 mL of Deparaffinization Buffer to the sample and vortex vigorously for 5 seconds.

   B2. Centrifuge briefly to immerse the sample in the Deparaffinization Buffer, 56℃ water bath for 5 minutes, intense vortex for 15 seconds.

   B3. Centrifuge for 2 minutes at 14,000 x g.

   B4. Thoroughly discard the supernatant, do not pipette up the precipitate. Proceed to Step 3.
3. Add 200 μL of Digestion Buffer and 20 μL of proteinase K to the sample, vortex and mix. 56℃ water bath for 1-2 hours until the sample is completely digested, during this period the mixture should be reversed up-and-down several times. The sample can also be digested overnight, with no negative effect on extraction results.
4. 90℃ water bath for 60 minutes.This step is to remove the cross-linking between DNA and protein and improve the yield of DNA.
5. **Optional step**: If the Digestion Buffer still contains significant undigested impurities, centrifuge at 10,000 x g for 3 minutes to remove the impurities. Transfer the supernatant to a new centrifuge tube. If RNA is to be removed, add 5 μL RNase A (100 mg/mL) to the supernatant and allow to stand for 5 minutes.
6. Add 200 μL of Binding Buffer and 200 μL of absolute ethanol to the sample, vortex and mix for 15 seconds. When processing multiple samples, the Binding Buffer and absolute ethanol can be premixed at a ratio of 1:1.

7. Place the Spin Column 4 in a collection tube, and transfer the mixed solution to the Spin Column 4. Centrifuge at 10,000 x g for 30 to 60 seconds.
8. Discard the filtrate, put the Spin Column 4 back into the collection tube, add 500 µL of Wash Buffer 1 to the column, and centrifuge at 10,000 x g for 30 to 60 seconds.
9. Discard the filtrate, put the Spin Column 4 back into the collection tube, add 650 µL of Wash Buffer 2 to the column, and centrifuge at 10,000 x g for 30 to 60 seconds.
10. Discard the filtrate, put the Spin Column 4 back into the collection tube, and centrifuge at 10,000 x g for 3 minutes.
11. Transfer the Spin Column 4 to a new 1.5 mL centrifuge tube, add 50 - 100 µL of Elution Buffer to **the center of the spin column membrane** . Leave for 1 minute at room temperature and centrifuge at 10,000 x g for 1 minute.

    Note: In order to improve the elution efficiency, 50 - 100 µL of Elution Buffer preheated at 56℃ can be added to the center of the spin column membrane, incubate at 56℃ for 5 min, and centrifuge at 10,000 x g for 1 min.
12. Discard the Spin Column 4, store the DNA at 2-8 ℃, and place it at -20 ℃ or -80 ℃ for long-term storage.

Simple scheme

J1. Transfer paraffin tissue sections (1 to 3 pieces) to 1.5 mL centrifuge tube. Add 0.6 mL of Deparaffinization Buffer to the sample and vortex vigorously for 5 seconds. Centrifuge briefly to immerse the sample in the Deparaffinization Buffer, 56℃ water bath for 5 minutes, intense vortex for 15 seconds.

J2. Centrifuge for 2 minutes at 14,000 x g to precipitate the tissue down to the tube bottom.

J3. Add 200 µL of Digestion Buffer and 20 µL of protease K to the bottom of the tube, gently pipetting mix 3 to 5 times, and centrifuge briefly to immerse the sample in the Digestion Buffer.

J4. 56℃ water bath for 60 minutes, 90℃water bath for 60 minutes.

J5. Centrifuge for 1 minute at 14,000 x g. Transfer the lower layer of fluid to a new centrifuge tube, and perform the operation according to Step 5 - 12 of the conventional scheme. (The upper layer is an organic phase formed by paraffin and dewaxing liquid.)

Note: Sample extraction efficiency depends on whether the operator strictly follows the instructions. False positive results may occur if cross contamination is not controlled during sample processing.