Quick Protocol

DNJia FFPE Tissue Kit

DNA isolation based on silica technology

- MiniPrep

For DNA Isolation from

Formalin-fixed, paraffin-embedded tissues

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Kit Content

Component	50 preps
TLB	9ml
GLB	10ml
BWB1 (concentrate)	16ml
BWB2 (concentrate)	16ml
RRB	5ml
RJ-Protease	2 X 1ml
HiPure DR Column	50
Collection Tube	50

Recommended Starting Material

Recommended to use freshly cut sections of FFPE tissue, each with a thickness of 5 to 10 µm. For each reaction, use up to 5 sections, each with a thickness of 5 to 10 µm and a surface area of up to 250 mm^2. If you are not sure about section properties use up to 3 sections for each reaction.

Before Start

- If GLB or TLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and will not influence the efficiency of buffer.
- Not forget to add the appropriate amount of ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation.

Washing Buffer Preparation

Before the first use, add appropriate amount of ethanol (96-100%) to each washing buffer tube, then mix thoroughly to prepare washing buffer, refer to Table 1. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by shaking. Storing at room temperature.

Table 1: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
BWB1	16ml	24ml	40ml
BWB1	16ml	24ml	40ml

Procedure of silica-based DNA isolation in quick look

Protocols

Protocol: ***Isolation of Genomic DNA (Formalin-fixed, paraffin-embedded tissues)***

Sample type: Formalin-fixed, paraffin-embedded tissues

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- If RNase treatment is desired, Prime-RNase A, can be ordered separately from ROJETechnologies, Cat No. EB983013.
- If TLB or GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and won't influence the efficiency of the buffer.
- Do not forget to add the appropriate amount of ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation part.

Process

- Trim excess paraffin off the sample block by using a scalpel. Cut the samples into sections 5-10 µm thick. Transfer up to 5 sections in 1.5 ml microcentrifuge tube.
- Add 1 ml xylene to the tube. Vortex vigorously for 30-60 s until paraffin becomes completely separated from the tissue and soluble in xylene. Centrifuge at 14000 rpm for 3 min.
- Remove the supernatant by pipetting. Do not remove any of the pellet.

  Attention! Do not disturb the pellet at the bottom of the tube.
- Add 1 ml ethanol (96-100%) to the pellet and mix by vortexing. Centrifuge at 14000 rpm for 3 min.
- Remove the supernatant by pipetting. Attention not to disturb the pellet.
- Repeat steps 4-5.
- Open the tube and incubate at room temperate for 10-15 min until the ethanol has evaporated thoroughly.
- Add 180 µl TLB. Mix by pulse vortexing for 30 s and then incubate at 56˚C for 10 min.
- Add 40 µl RJ-Protease to the sample. Mix thoroughly by pulse vortexing for 30 s and then incubate at 56˚C for 1-2 h. Pulse vortex every 10 min during incubations to intersperse the sample, or place it in a thermomixer or shaking water bath.

  Note: you might observe white precipitates in the lysate during lysis, these white precipitates will not disappear and you must apply them to the column.

Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10 µl Prime-RNase A (Cat No. EB983013), mix by vortexing, and incubate for 5 min at room temperature before going to step 3.

- Add 200 µl GLB to the mixture, mix by pulse vortexing for 10 s and then incubate at 70°C for 10 min.
- Add 200 µl absolute ethanol, then mix thoroughly by pulse vortexing for 15 s.
- Pipette the mixture from step 11 to a HiPure DR Column in a 2 ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1 min. Discard flow-through and place the spin column in the collection tube again.

  Note: Apply the white precipitate onto the column. If the lysate did not pass the column, centrifuge at full speed until it passes through the column.
- Add 700 µl BWB1 to the HiPure DR Column. Centrifuge at 13000 rpm for 1 min, just discard the flow-through. Place the HiPure DR Column in the previous collection tube and go to the next step.
- Add 600 µl BWB2 to the HiPure DR Column. Centrifuge at 14000 rpm for 3 min. Discard both the flow-through and the collection tube. Place the HiPure DR Column in a new clean 1.5 ml microcentrifuge tube (not provided).

  Note: To avoid ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1 min at 14000 rpm.
- Pipette 50-100 µl RRB directly onto HiPure DR Column. Incubate at room temperature for 5 min. Then Centrifuge it at 13000 rpm for 1 min.