SECTION 1. DEPARAFFINIZATION OF FFPE SAMPLES

1.1 Deparaffinization of Tissue

The maximum recommended input is 4 sections of ≤20µm thickness. Alternatively, an unsectioned core of up to 10mg may be used.

1.1.1 Protocol

1. Cut 4 sections up to 20µm thick from the interior of an FFPE tissue block using a microtome. Trim off any excess paraffin.
   Note: Alternatively, cut out up to 10mg of unsectioned core from an FFPE block. Trim off any excess paraffin. Grind the sample into fine powder using liquid nitrogen.
2. Transfer the sections or ground block into a 1.5ml RNase-free microcentrifuge tube.
3. Add 1ml of xylene (user supplied) to the sample. Mix by vortexing. Incubate at 50°C for 5 min.
4. Centrifuge sample for 2 min at 14,000 x g to pellet the tissue. Carefully remove and discard the xylene without dislodging the pellet.
5. Add 1ml of 96-100% ethanol. Mix by vortexing.
6. Centrifuge the sample for 2 min at 14,000 x g. Carefully remove and discard the ethanol without dislodging the pellet.
7. Repeat steps 5 to 6.
8. Air-dry the pellet for about 10 min at room temperature. **Proceed to section 2.**
   Note: It is important to remove the ethanol completely.

SECTION 2. TOTAL RNA PURIFICATION

2.1 Lysate Preparation

1. Add 300µl of Digestion Buffer DX and 10µl of the reconstituted Proteinase K solution to the sample. Mix by vortexing.
2. Incubate at 55°C for 15 min. Vortex occasionally.
3. Cool the sample on ice for 3 min.
4. Centrifuge the sample for 3 min at 14,000 x g.
5. Carefully transfer the RNA-containing supernatant to a new 1.5ml RNase-free microcentrifuge tube (user supplied). **Retain the microcentrifuge tube containing the pellet for DNA purification.**
   Note: The DNA-containing pellet can be stored for 2 hours at room temperature, for up to 24 hours at 4°C, or at –20°C for longer-term storage.

6. Incubate the tube of the RNA-containing supernatant at 80°C for 15 min. Vortex occasionally.
   Note: Do not exceed 15 min of incubation at 80°C as this will increase RNA fragmentation.
7. Add 300µl of Lysis Buffer RX. Vortex for 3s to mix.
8. Add 600µl of 96–100% ethanol. Vortex for 3s to mix.

2.2 Binding RNA to Column

1. Assemble an ISOLATE II **FFPE RNA Micro Column** (black ring) with a Collection Tube (supplied).
2. Apply up to 600µl of the ethanolic lysate (from section 2.1, step 8) onto the column and centrifuge for 1 min at ≥ 3,500 x g.
   Note: Ensure the entire lysate volume has passed through into the Collection Tube by inspecting the column. If the entire lysate volume has not passed through, centrifuge for an additional 1 min at 14,000 x g.
3. Discard the flow-through. Reassemble the spin column with its Collection Tube.
4. Repeat steps 2 and 3 until all lysate has passed through the column.
5. **Optional:** The ISOLATE II FFPE RNA/DNA Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, for sensitive applications, an optional on-column DNA removal protocol is provided (see full manual Appendix A). DNase I treatment should be performed at this point in the protocol with the supplied DNase I and reaction buffer.

2.3 RNA Column Wash

1. Apply 400µl Wash Buffer W1 to the column and centrifuge for 1 min at 14,000 x g.
2. Discard the flow-through and reassemble the spin column with its Collection Tube.
   Note: Ensure the entire wash buffer has passed through into the Collection Tube by inspecting the column. If the entire wash volume has not passed through, centrifuge for an additional 1 min at 14,000 x g.
3. Apply 400µl Wash Buffer W1 to the column and centrifuge for 1 min at 14,000 x g.
4. Discard the flow-through and reassemble the spin column with its Collection Tube.
5. Wash the column a third time by adding 400µl of Wash Buffer W1 to the column and centrifuge for 1 min at 14,000 x g. Discard the flow-through and reassemble the spin column with its Collection Tube.
6. Centrifuge for 2 min at 14,000 x g in order to dry the column thoroughly. Discard the Collection Tube.

2.4 **RNA Elution**

1. Place the column into a fresh 1.7ml Elution Tube (supplied).
2. Add 20-50µl of RNA Elution Buffer to the column.
   Note: If highly concentrated RNA is required, as little as 15µl of RNA Elution Buffer can be used. However, the RNA yield may be reduced when a smaller elution volume is used.
3. Centrifuge for 2 min at 200 x g, followed by 1 min at 14,000 x g. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column for an additional 1 min at 14,000 x g.
   Optional: For maximum RNA recovery, apply a second volume of RNA Elution Buffer and repeat step 3. Alternatively, re-apply the first eluate onto the column and re-elute for high concentration.

2.5 **Storage of RNA**

Store isolated RNA at –20°C for up to three days or at -80°C (recommended) for long term storage.

SECTION 3. GENOMIC DNA PURIFICATION

3.1 **Lysate Preparation**

1. Add 300µl of Digestion Buffer DX and 10µl of Proteinase K solution to the DNA-containing pellet (obtained from section 2.1, step 5). Mix by vortexing.
2. Incubate at 55°C for 1 hour. Vortex occasionally to mix.
3. Incubate at 90°C for 2 hours. Vortex gently occasionally to mix.
   Note: This step is necessary to reverse formalin cross-links on the DNA resulting from the fixative process. Reducing the incubation time may result in the DNA not performing optimally in downstream applications due to the formalin cross-links not being completely removed.
4. Cool the sample on ice for 3 min.
   Note: The ISOLATE II FFPE RNA/DNA Kit isolates DNA with minimal amounts of RNA contamination. However, if it is required to remove any trace amount of RNA, add 4µl of RNase A (10mg/ml) (user supplied) to the cooled lysate and incubate at room temperature for 5 min.
5. Add 300µl of Lysis Buffer RX. Vortex for 3s to mix.
6. Add 250µl of 96–100% ethanol. Vortex for 3s to mix.

3.2 **Binding DNA to Column**

1. Assemble an ISOLATE II **FFPE DNA Micro Column** (white ring) with a Collection Tube (supplied).
2. Apply up to 600µl of the ethanolic lysate onto the column (from section 3.1, step 6) and centrifuge for 1 min at 14,000 x g.
3. Discard the flow-through. Reassemble the spin column with its Collection Tube.
4. Repeat steps 2 and 3 until all lysate has passed through the column.

3.3 DNA Column Wash

1. Apply 600µl of Wash Buffer W1 to the column and centrifuge for 1 min at 14,000 x g.
   Note: Ensure the entire wash buffer has passed through into the Collection Tube by inspecting the column. If the entire wash volume has not passed through, spin for an additional 1 min at 14,000 x g.
2. Discard the flow-through and reassemble the spin column with its Collection Tube. Apply 600µl of Wash Buffer W1 to the column and centrifuge for 1 min at 14,000 x g.
3. Discard the flow-through and reassemble the spin column with its Collection Tube.
4. Wash column a third time by adding 600µl of Wash Buffer W1 and centrifuge for 1 min at 14,000 x g.
5. Discard the flow-through and reassemble the spin column with its Collection Tube.
6. Centrifuge column for 2 min at 14,000 x g in order to dry the column thoroughly. Discard the Collection Tube.

3.4 DNA Elution

1. Place the column into a fresh 1.7ml Elution Tube (supplied).
2. Add 20-50µl of DNA Elution Buffer to the column. Incubate the assembly at room temperature (18-25°C) for 1 min.
3. Centrifuge column for 1 min at 14,000 x g. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column for an additional 1 min at 14,000 x g.
   Optional: For maximum DNA recovery, apply a second volume of DNA Elution Buffer and repeat step 3. Alternatively, re-apply the first eluate onto the column and re-elute for higher concentration.

3.5 Storage of DNA

Store isolated DNA at 4°C for up to three days. It is recommended that samples are placed at -20°C or -80°C for long term storage.

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II FFPE RNA/DNA Kit (Phenol free)	50 Preps	BIO-52087

Technical Support
The troubleshooting guide is provided in the online protocol.
For technical support please email us at tech@bioline.com
or visit www.bioline.com

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