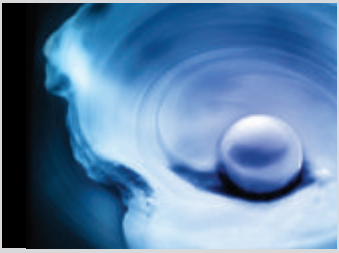


ISOLATE II
FFPE RNA/DNA Kit
(Phenol free)

Product Manual

BIOLINE

A Meridian Life Science® Company

ISOLATE II FFPE RNA/DNA Kit (Phenol free)

1. KIT CONTENTS

COMPONENT	50 Preps
ISOLATE II FFPE RNA Micro Columns (black)	50
ISOLATE II FFPE DNA Micro Columns (white)	50
Collection Tubes (2ml)	100
Elution Tubes (1.7ml)	100
Proteinase K (lyophilized)	2 x 12mg
Digestion Buffer DX	2 x 25ml
Lysis Buffer RX*	40ml
Wash Buffer W1† (Concentrate)	2 x 38ml
RNA Elution Buffer	6ml
DNA Elution Buffer	15ml
DNase I Solution	210µl
DNase I Reaction Buffer DRB	6ml
Product Manual	1
Bench-Top Protocol	1

* *Contains a guanidine salt. Not compatible with disinfectants containing bleach or acidic solutions. See safety information in section 4.*

† *Before use, add 90ml of 96-100% ethanol and mark wash buffer bottle label.*

2. DESCRIPTION

The ISOLATE II FFPE RNA/DNA Kit provides a rapid phenol-free method for the sequential isolation and purification of total RNA (including microRNA) and genomic DNA from formalin-fixed paraffin-embedded (FFPE) tissue samples. Formalin fixation of tissues results in crosslinking of the nucleic acids and proteins. Furthermore, the embedding process can also lead to fragmented nucleic acids with time. The ISOLATE II FFPE RNA/DNA Kit has been developed to allow for partial reversing of formalin modifications, resulting in high quality and yield of nucleic acids.

Isolation is based on a fast spin column format using a novel affinity resin as the separation matrix so that the RNA and DNA are preferentially purified from other cellular components. This kit does not require the use of phenol or chloroform.

FFPE samples are first deparaffinized with xylene and ethanol wash steps, then digested with the supplied Proteinase K and Digestion Buffer. Solubilized lysate is collected for RNA purification, whilst the remaining sample is further digested for DNA. Lysis buffer and ethanol are added to the RNA or DNA lysates and loaded onto a RNA or DNA Micro Column respectively. Bound nucleic acids are washed to remove any contaminants. The purified RNA is of the highest integrity and can be used in a number of downstream applications including real-time PCR, reverse transcription PCR, RNA-seq, expression arrays, Nanostring and Fluidigm assays. The purified genomic DNA is also of the highest quality and can be used in real-time PCR, PCR, next generation sequencing, Southern blotting and SNP analysis.

Please read this manual carefully to familiarize yourself with the ISOLATE II FFPE RNA/DNA protocol before starting (also available on www.bioline.com/isolate). More experienced users can refer to the Bench-Top Protocol for quick referencing during the procedure.

3. STORAGE

The DNase I and Proteinase K should be stored at -20°C upon arrival. All other components should be stored at room temperature (18-25°C). Storage at lower temperatures may cause precipitation of salts.

4. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Lysis Buffer RX contains guanidinium thiocyanate. This chemical is harmful in liquid form when in contact with skin or ingested. If the solution is allowed to dry, the powder is harmful if inhaled.

CAUTION: Do not add bleach directly to solutions or sample preparation waste containing guanidinium salts. Reactive compounds and toxic gases can form. In the case of spillage, clean the affected area with a suitable laboratory detergent and water.

For detailed information, please consult the material data safety sheet (MSDS) available on our website at www.bioline.com.

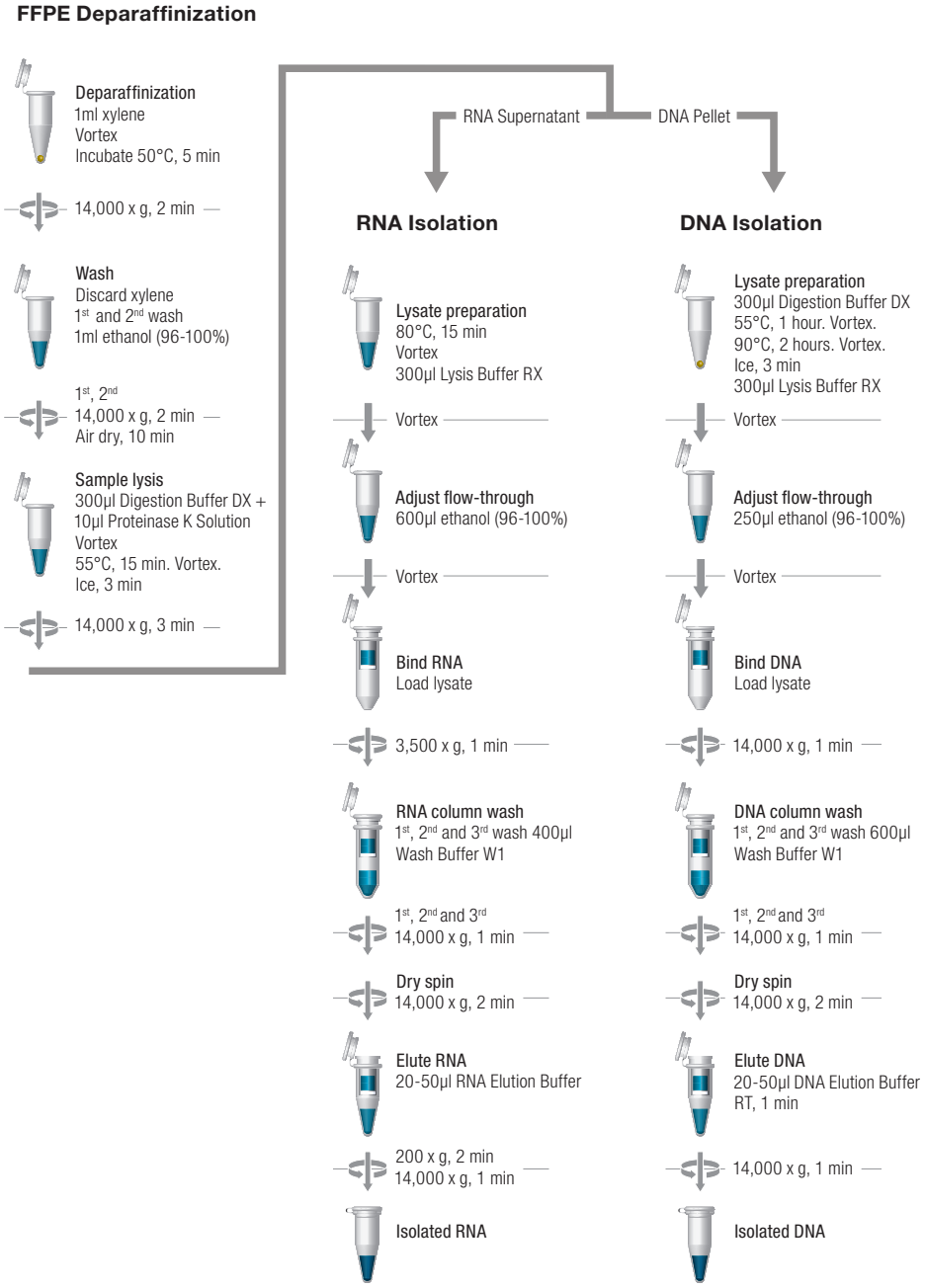
5. PRODUCT SPECIFICATIONS

The ISOLATE II FFPE RNA/DNA Kit is specially designed to purify all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). RNA size will depend on the age of the FFPE tissue as the degree of fragmentation of the RNA will increase over time. Both the purified total RNA and genomic DNA are of the highest quality and are ready to use in various downstream applications.

ISOLATE II FFPE RNA/DNA MICRO COLUMN SPECIFICATIONS	
Max. binding capacity	35µg for RNA 10µg for DNA
Max. column loading volume	600µl
RNA size distribution	All sizes, including small RNA <200 nucleotides
DNA size distribution	All sizes >80bp
Max. amount of starting material	4 sections <20µM thick 10mg of unsectioned block

The following component is also included in the kit:

- DNase I (RNase free) for eliminating genomic DNA contamination by on-column digestion or by digestion in solution (for the most sensitive applications).



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

When working with chemicals, always wear a suitable lab coat, protective goggles and disposable gloves. Please consult the relevant MSDS from the product supplier for further information and see section 4.

The following may be supplied by the user:

- ß-mercaptoethanol (ß-ME)* (Optional for Lysis Buffer RX)
- 96-100% ethanol† (for Wash Buffer W1)
- Xylene, histological grade
- Equipment for sample disruption and homogenization (see section 7.2). The following are required if using unsectioned cores from FFPE tissue blocks:
  - o Mortar and pestle
  - o Liquid nitrogen
- Molecular biology grade water
- RNase-free microcentrifuge tubes (1.5ml)
- RNase-free tips
- Benchtop microcentrifuge (capable of 14,000 x g)
- Incubator or water bath (capable of 50-55°C, 80°C and 90°C)

** TCEP is also a suitable reducing agent instead of ß-ME.*
† Molecular biology grade ethanol is recommended. Do not use denatured alcohol which contains unwanted additives such as methanol and acetone.

7. IMPORTANT NOTES

The protocol steps are deparaffinization of the FFPE sample, purification of total RNA and purification of genomic DNA. Optional DNase I treatment protocols are provided.

- Section 8 contains the FFPE sample deparaffinization protocol.
- Section 9 contains the protocol to purify total RNA.
- Section 10 contains the protocol to purify genomic DNA.
- The Appendix contains the optional protocols for on-column or in-solution DNase I treatment.

The ISOLATE II FFPE RNA/DNA Kit purification procedures can be performed at room temperature. Handle the eluted RNA carefully to avoid contamination by RNases, often found on labware, fingerprints and dust. For optimal RNA stability, keep RNA frozen at -20°C for short-term or -80°C for long-term storage. When working with RNA samples in downstream applications, keep the RNA solution on ice.

Two types of spin columns are provided with this kit: the ISOLATE II **FFPE RNA Micro Column** (black ring) and the ISOLATE II **FFPE DNA Micro Column** (white ring). Ensure the correct column is used for each step of the procedure.

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g except where noted. Perform all centrifugation steps at room temperature.

Ensure that all solutions are at room temperature prior to use.

It is important to work quickly when purifying RNA (see hints and tips on working with RNA at www.bioline.com/isolate).

7.1 HANDLING AND STORING STARTING MATERIALS

Formalin-fixed paraffin embedded samples are an invaluable biospecimen resource for both diagnostic histopathology and molecular studies. The quality and yield of extracted nucleic acids isolated from FFPE specimens can be affected by a number of factors including: upstream tissue collection, sample type, fixation, age and storage conditions.

We recommend the following guidelines to improve recovery of intact nucleic acids from FFPE samples:

7.1.1 TISSUE COLLECTION AND FIXATION

Tissues should be fixed within one hour of surgical resection. The optimal fixation time is 12–24 hours, using neutral-buffered formalin or paraformaldehyde. Extensive degradation of RNA can occur before completion of the fixation process. Attention should be paid to the time of fixation so as not to cause overfixation or underfixation. Fixed tissues should be thoroughly dehydrated prior to the embedding process.

Fixing tissues with formalin leads to crosslinking of nucleic acids and proteins which impairs RNA or DNA performance in downstream applications. The ISOLATE II FFPE RNA/DNA Kit provides special lysis and incubation conditions to reverse formalin crosslinking of nucleic acids.

7.1.2 SECTIONING

Prior to sectioning, the microtome and accessories should be cleaned with RNase-denaturing reagents. FFPE tissue samples for RNA extraction should be taken from a previously unexposed portion of the tissue block as oxidation can rapidly degrade RNA from exposed margins. It is good practice to trim the face of the tissue block prior to cutting the samples that will be used for RNA isolation by cutting and discarding several 10-micron thick sections prior to cutting the sample sections.

7.1.3 STORAGE

For optimal preservation, FFPE tissue should be stored as a block and not sliced until analysis is imminent. Blocks should be stored without cut faces to prevent ongoing damage from exposure to oxygen, water and other environmental factors such as light and infestation.

Studies show that under normal storage conditions, nucleic acids in FFPE specimens are relatively stable for extended periods (Xie *et al*., 2011). However, it should be noted that some studies show degradation of nucleic acids in FFPE blocks over periods of months to years will occur under even the best storage conditions (Engel & Moore 2011; Xie *et al.* 2011). Degradation of RNA can occur within FFPE blocks over time and is well documented (Cronin *et al*., 2004; Hewitt *et al*., 2008). Therefore, some FFPE-derived nucleic acid samples may be too severely compromised for use in gene expression analysis or other applications.

7.2 DISRUPTING AND HOMOGENIZING STARTING MATERIAL

7.2.1 Disruption of unsectioned core samples using a mortar and pestle

An RNase-free mortar and pestle can be used in combination with liquid nitrogen to disrupt the tissue from an unsectioned core of a FFPE block. Up to 10mg of an unsectioned core sample can be used. Trim off the excess paraffin. Add liquid nitrogen to the tissue in the unsectioned core and grind the frozen tissue into a fine powder. After grinding, transfer tissue powder into a tube and proceed with deparaffinization. Refer to section 8.1.

7.3 BUFFER PREPARATION AND PARAMETERS

Ensure 96-100% ethanol is available. Prepare the following:

7.3.1 Preparing Wash Buffer W1 with ethanol

Add 90ml of 96-100% ethanol to the 38ml of Wash Buffer W1 Concentrate in each of the supplied bottles to give a final volume of 128ml.

Note: Mark the label of the bottle to indicate ethanol was added. Wash Buffer W1 is used for both RNA and DNA Purification. Store Wash Buffer W1 at room temperature (18-25°C).

7.3.2 Preparing Proteinase K stock solution

Reconstitute each vial of lyophilized Proteinase K by adding 600µl molecular biology grade water (user supplied). Incubate for 1 min at room temperature, then mix by gently swirling. Dispense into small aliquots to avoid excessive freeze-thawing. Store aliquots at -20°C.

7.3.3 Preparing Lysis Buffer RX with ß-mercaptoethanol (optional)

Optional: The use of ß-mercaptoethanol (ß-ME) in lysis is highly recommended for most mammalian tissues, particularly those known to have a high RNase content (e.g. pancreatic tissue). It is also recommended for users who wish to isolate RNA for sensitive downstream applications or target transcripts of low quantity. Add 10µl of ß-ME (provided by the user) to each 1ml of Buffer RX required. ß-ME is toxic and should be dispensed in a fume hood. Alternatively, Buffer RX can be used as provided.

Note: TCEP can also be used as an alternative reducing agent. Use TCEP at a final concentration of 10mM within Lysis Buffer RX.

7.3.4 Preparing DNase I (RNase-free) (optional)

Optional on-column digestion: For each on-column reaction to be performed, prepare a mix of 4µl of DNase I and 96µl of DNase Reaction Buffer DRB. Mix gently by inverting a few times.

Optional in-solution digestion: In a microcentrifuge tube, mix together 10µl of DNase Reaction Buffer DRB, 2.5µl of DNase I and up to 87.5µl of RNA solution. For lower starting volumes of RNA, bring the volume up to 100µl using RNase-free water. Gently swirl tube to mix solution.

Note: Do not vortex the DNase I as the enzyme is particularly sensitive to mechanical denaturation. Dispense into aliquots to avoid excessive freeze-thawing. Store aliquots at -20°C.

7.3.5 Elution parameters

Elute DNA or RNA using the provided DNA or RNA Elution Buffers, respectively. The standard elution protocol can be modified for different applications.

- To achieve high yield, perform two successive elution steps with an elution volume described in the individual protocol (90-100% recovery rate). You may elute into the same or a different microcentrifuge tube depending on your application.
- For both high-yield and high-concentration, elute with the standard elution volume. Then re-apply eluate onto the column for re-elution into the same microcentrifuge tube.

Eluted DNA may be stored at 4°C for a few days. For short-term storage freeze at -20°C, but for long-term storage, freeze at -80°C.

Always place eluted RNA on ice immediately to prevent degradation by RNases. For short-term storage freeze at -20°C. For long-term storage, freeze at -80°C.

7.4 ELIMINATING GENOMIC DNA CONTAMINATION

For most applications, genomic DNA is undetectable in preparations of RNA using the ISOLATE II FFPE RNA/DNA Kit. Genomic DNA contamination is efficiently removed by on-column digestion with DNase I (see section 7.3.4 and Appendix A). However, residual genomic DNA contamination may be detected in very sensitive applications e.g. probe-based real-time PCR. A DNase I digest in the eluate can be performed to remove even traces of contaminating DNA (see section 7.3.4 and Appendix B).

8. DEPARAFFINIZATION OF FFPE SAMPLES

This protocol is designed for removal of paraffin from FFPE tissue samples. The maximum recommended input is four individual sections of ≤20µm thickness. It is important to obtain sections from the interior of an FFPE block in order to minimize RNA or DNA damage by oxidation.

Alternatively, an unsectioned core sample (up to 10mg) from a FFPE block may be used (see section 7.2.1).

After pelleting of the sample and removal of the supernatant, residual xylene is removed by washing with ethanol.

8.1 Deparaffinization of Tissue

1. Cut four 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 a FFPE block. Trim off any excess paraffin. Grind the sample into fine powder using liquid nitrogen. Refer to section 7.2.1 on disruption using a mortar and pestle.
2. Transfer the sections or ground block into an 1.5ml RNase-free microcentrifuge tube.
3. Add 1ml of xylene to the sample. Mix by vortexing. Incubate at 50°C for 5 min.
4. Centrifuge 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 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 9**
   Note: It is important to remove the ethanol completely.

9. TOTAL RNA PURIFICATION

Before you start:

- Ensure Lysis Buffer RX, Proteinase K and Wash Buffer W1 are prepared (see section 7.3).
- Ensure you use the ISOLATE II FFPE **RNA Micro Column** (black ring) for this procedure.

9.1 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 24 hours at 4°C, or at -20°C for longer term storage.
6. Incubate the tube of the RNA-containing lysate 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.

9.2 Binding RNA to Column

1. Assemble an ISOLATE II **FFPE RNA Micro Column** (black ring) with a Collection Tube (provided).
2. Apply up to 600µl of the ethanolic lysate (from section 9.1 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 minute 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 in Appendix A. DNase I treatment should be performed at this point in the protocol with the supplied DNase I. For highly sensitive applications, in-solution DNase treatment can be performed (see Appendix B).

9.3 RNA Column Wash

1. Apply 400µl of 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 minute at 14,000 x g.
3. Apply 400µl of 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 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 the column for 2 min at 14,000 x g in order to dry the column thoroughly. Discard the Collection Tube.

9.4 RNA Elution

1. Place the column into a sterile 1.7ml Elution Tube.
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 minute at 14,000 x g.

   Note: For maximum RNA recovery, it is recommended to apply a second volume of RNA Elution Buffer followed by elution into the same microcentrifuge tube (repeat steps 2 and 3). Alternatively, re-apply the first eluate onto the column and re-elute into the same microcentrifuge tube (for high concentration).

9.5 Storage of RNA

The isolated RNA can be stored at -20°C for up to three days or at -80°C for long-term storage.

10. GENOMIC DNA PURIFICATION

Before you start:

- Ensure Lysis Buffer RX, Proteinase K and Wash Buffer W1 are prepared (see section 7.3).
- Ensure you use the ISOLATE II FFPE **DNA Micro Column** (white ring) for this procedure.

Optional reagent required:

- RNase A (10mg/ml)

10.1 Lysate Preparation

1. Add 300µl of Digestion Buffer DX and 10µl of the reconstituted Proteinase K solution to the DNA-containing pellet obtained from section 9.1, 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 incubation 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.

10.2 Binding DNA to Column

1. Assemble an ISOLATE II **FFPE DNA Micro Column** (white ring) with a Collection Tube (provided).
2. Apply up to 600µl of the ethanolic lysate (from section 10.1, step 6) onto the column 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.

10.3 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 minute 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 the 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 the column for 2 min at 14,000 x g in order to dry the column thoroughly. Discard the Collection Tube.

10.4 DNA Elution

1. Place the column into a fresh 1.7ml Elution Tube.
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 minute at 14,000 x g.

   Note: For maximum DNA recovery, it is recommended to apply a second volume of DNA Elution Buffer followed by elution into the same microcentrifuge tube (repeat steps 2 and 3). Alternatively, re-apply the first eluate onto the column and re-elute into the same microcentrifuge tube (for higher concentration).

10.5 Storage of DNA

The isolated DNA can be stored at 4°C for a few days. It is recommended that samples are placed at -20°C or -80°C for long-term storage.

11. APPENDICES

11.1 APPENDIX A: OPTIONAL ON-COLUMN DNASE I TREATMENT PROTOCOL

The ISOLATE II FFPE RNA/DNA Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications.

11.1.1 Protocol

1. For each on-column digest to be performed, prepare a DNase I - buffer mix by adding 4µl of the supplied DNase I Solution to 96µl of DNase I Reaction Buffer DRB. Mix gently by inverting the tube a few times. Do not vortex.
2. Apply 400µl of Wash Buffer W1 to the **FFPE RNA Micro Column** (black ring) and centrifuge for 2 min at 14,000 x g. Discard the flow-through. 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, spin for an additional minute at 14,000 x g.

3. Apply 100µl of the DNase I - buffer mix to the column and centrifuge for 1 min at 14,000 x g.

   Note: Ensure the entire volume of DNase I - buffer mix passes through the column. If needed, spin for an additional minute at 14,000 x g.

4. Pipette the flow-through present in the Collection Tube back onto the top of the column.

   Note: This step must be performed in order to ensure maximum DNase I activity and to obtain maximum yields of RNA. This is particularly important for the isolation of small RNA species.

5. Incubate at room temperature (18-25°C) for 15 min. Without any further centrifugation, proceed directly to the second wash step in the RNA Column Wash section (section 9.3, step 3). Apply the wash buffer directly to the column containing the DNase I - buffer mix.

11.2 APPENDIX B: OPTIONAL DNASE I TREATMENT OF PURIFIED RNA IN SOLUTION PROTOCOL

The on-column DNase I digestion results in minimal residual DNA, undetectable in most downstream applications. For the most sensitive applications, DNA digestion in solution is recommended to eliminate even traces of contaminating DNA. Careful control of RNase contamination is needed, as well as RNA repurification to remove buffer, salts, DNase I or digested DNA.

Additional reagents required:

- Sodium acetate (3M, pH 5.2)
- Ice-cold 70% ethanol

11.2.1 Protocol

1. In a RNase-free 1.5ml microcentrifuge tube (user supplied), mix together 2.5µl of the supplied DNase I Solution, 10µl of DNase I Reaction Buffer DRB, and up to 87.5µl of eluted RNA. If using a lower starting volume of RNA solution, bring the volume up to 100µl using RNase-free water.
2. Gently swirl tube to mix solution. Gently spin down (approx. 1s at 1000 x g) to collect solution at the bottom of tube.
3. Incubate at room temperature (18-25°C) for 10 min.
4. Repurify the RNA with a suitable RNA clean-up procedure, e.g. using ethanol precipitation.

Ethanol precipitation step

- Add 1/10th volume of sodium acetate (3M, pH 5.2).
- Add between 2.5 and 3 volumes of 96-100% ethanol to one volume of sample. Mix thoroughly.
- Precipitate for one hour at -20°C or overnight at -20°C.

  Note: Choose longer incubation times if the sample has a low RNA concentration. Shorter incubation times are sufficient for high RNA concentrations.

- Centrifuge at maximum speed for 10 min.
- Wash the RNA pellet with ice-cold 70% ethanol.
- Dry the RNA pellet and resuspend the RNA in RNase-free water.

12. TROUBLESHOOTING GUIDE

LOW RNA YIELD OR QUALITY	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Incomplete lysis	Ensure appropriate amount of Digestion Buffer DX and Proteinase K was used. Increase incubation time.
Ethanol or Buffer RX was not added to the lysate	Ensure appropriate amount of ethanol and Lysis Buffer RX is added to lysate/flowthrough before binding to column.
Ethanol not added to the Wash Buffer	Ensure 90ml of 96-100% ethanol is added to Wash Buffer W1 prior to use.
NUCLEIC ACID DOES NOT PERFORM WELL IN DOWNSTREAM APPLICATIONS	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Nucleic acids not washed three times with Wash Buffer	Traces of salt from binding step may remain in the sample if column is not washed three times with Wash Buffer W1.
Ethanol carryover during elution	Increase centrifugation time for ethanol removal step. Ensure final dry spin under Column Wash procedure is performed, in order to remove traces of ethanol prior to elution.
Formalin crosslinks not completely reversed	Ensure sufficient incubation at 80°C or 90°C is performed to remove formalin crosslinks.

CLOGGED SPIN COLUMN	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Insufficient solubilization	Ensure correct amount of Digestion Buffer DX with Proteinase K was used. Increase incubation time.
Maximum number of sections or amount of starting material exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications.
Centrifuge temperature too low	Ensure centrifuge remains at room temperature throughout procedure. Temperatures below 15°C may cause precipitates to form that can cause columns to clog.
Insufficient centrifugation	Increase centrifugation speed and time.
RNA DEGRADED	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
FFPE sample is old	Quality of RNA purified may drastically decrease in old samples. For best performance, freshly prepared samples are highly recommended.
RNase contamination	Ensure an RNase-free working environment (see online hints and tips at www.bioline.com/isolate). Discard any solutions contaminated with RNase during use.
Too long incubation of lysate at high temperature	In order to maintain RNA integrity, it is important that the procedure is performed according to the time indicated. This is especially important for the lysate preparation step in section 9.1 when the sample is incubated at 55°C and 80°C for 15 min each.
Improper storage of purified RNA	For short term storage RNA samples may be stored at -20°C for up to three days. It is recommended that samples are kept at -80°C for longer term storage.
Starting material may have a high RNase content	For starting materials with high RNase content, it is recommended that ß-ME or TCEP is added to Lysis Buffer RX.
GENOMIC DNA CONTAMINATION	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
DNase I inactive	Store as recommended.
Large amounts of starting material used	Perform DNase I digestion to remove any residual genomic DNA contamination (see Appendix A and B).
On-column DNase I digestion step not performed	Perform on-column DNase I treatment protocol provided (see Appendix A).
Residual genomic DNA contamination remaining after on-column DNase I digest performed	Perform in-solution DNase I treatment protocol provided (see Appendix B) to eliminate traces of contaminating genomic DNA. In solution DNase I digestion is recommended for most sensitive downstream applications.

A. TECHNICAL SUPPORT

For technical assistance or more information on these products, please email us at tech@bioline.com

B. ORDERING INFORMATION

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

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
SensiFAST™ cDNA Synthesis Kit	50 Reactions	BIO-65053
SensiFAST™ Probe No-ROX Kit	200 Reactions	BIO-86002
MyTaq™ HS DNA Polymerase	250 Units	BIO-21111

D. PRODUCT WARRANTY AND DISCLAIMER

Bioline warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Bioline will replace any product that does not conform to the specifications. The warranty limits Bioline's liability to only the replacement of the product.

A Meridian Life Science® Company

www.bioline.com

Australia

Bioline (Aust) Pty Ltd
Tel: +61 (0)2 9209 4180
email: info.aust@bioline.com

France

Bioline France
Tel: +33 (0)1 42 56 04 40
email: orders.fr@bioline.com

Germany

Bioline GmbH
Tel: +49 (0)3371 68 12 29
email: info.de@bioline.com

Singapore

Bioline (Meridian Bioscience Asia Pte. Ltd.)
Toll Free: 1800 BIOLINE (2465463)
Tel: +65 6774 7196
email: info.sg@bioline.com

United Kingdom

Bioline Reagents Ltd
Tel: +44 (0)20 8830 5300
email: info.uk@bioline.com

USA

Bioline USA Inc.
Order Toll Free: +1 888 257 5155
Tel: +1 508 880 8990
email: info.us@bioline.com