RecoverAll™ Multi-Sample RNA/DNA Workflow

Optimized workflow for sequential extraction of RNA and DNA from FFPE samples

Catalog Numbers A26069 and A26135

Pub. No. MAN0010642 **Rev.** D.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Workflow information

The Invitrogen™ RecoverAll™ Multi-Sample RNA/DNA Workflow is a complete workflow for sequential extraction of RNA and DNA from the same sample. The recovered RNA and DNA are ready for use with downstream applications such as quantitative real-time RT-PCR and targeted sequencing that is compatible with the Oncomine® Cancer Research Panel.

The workflow utilizes the proven technology of the RecoverAll™ Total Nucleic Acid Isolation Kit for extraction of RNA and DNA from formaldehyde- or paraformaldehyde-fixed, paraffin-embedded (FFPE) tissues. A maximum of four 20-µm sections can be processed per reaction.

First, FFPE slide-mounted or tissue blocks sections are deparaffinized and digested with Protease. The samples are then loaded on a PureLink™ column; DNA remains bound to the column while RNA flows through. To recover RNA, the flow-through is applied to a new column, treated with DNase, then eluted from the filter. To recover DNA, the DNA bound to the column is eluted.

Provided materials and storage

Cat. Nos. A26069 and A26135 provide three off-the-shelf products used in the workflow and are used in conjunction with the PureLink™ RNA Mini Columns (Cat. No. A29839; 3 and 4 bags for Cat. Nos. A26069 and A26135, respectively) and the PureLink™ Viral Collection Tubes (Cat. No. 12282100; 1 and 2 bags for Cat. Nos. A26069 and A26135, respectively) .

Table 2 Provided materials for the RecoverAll™ Multi-Sample RNA/DNA Workflow

Components	Cat. No. A26069[1]	Cat. No. A26135[2]	Storage
RecoverAll™ Total Nucleic Acid Isolation Kit[3] (Cat. no. AM1975)	3 kits	5 kits	See Table 3
Elution Tubes (2.0 mL) (Cat. no. AM12480)	100 tubes	100 tubes	15°C to 30°C
Nuclease-Free Water (Not DEPC-Treated) (Cat. no. AM9938)	100 mL	100 mL	

[1] Sufficient for 60 RNA isolations and 60 genomic DNA isolations.
[2] Sufficient for 100 RNA isolations and 100 genomic DNA isolations.
[3] See Table 3 for kit contents.

Table 3 RecoverAll™ Total Nucleic Acid Isolation Kit (Cat. no. AM1975) contents and storage

Component	Amount	Storage
Digestion Buffer	16 mL	15°C to 30°C
Isolation Additive	19.2 mL	
Wash 1 Concentrate	60 mL[1]	
Wash 2/3 Concentrate	60 mL[1]	
Protease	160 µL	–15°C to –25°C
Elution Solution	5 mL	15°C to 30°C
DNase	160 µL	–15°C to –25°C
10X DNase Buffer	240 µL	
Filter Cartridges [2]	40 cartridges	15°C to 30°C
Collection Tubes[2]	80 tubes	
RNase A[2]	400 µL	–15°C to –25°C

[1] Final volume; see "Before first use of the kit: prepare buffers" on page 3.
[2] Not used in this worklfow.

Note: We recommend using the PureLink™ RNA Mini Columns (Cat. No. A29839) and the PureLink™ Viral Collection Tubes (Cat. No. 12282100) in this workflow, instead of the Filter Cartridges and Collection Tubes contained in the kit. Using PureLink™ RNA Mini Columns and the PureLink™ Viral Collection Tubes allows for a lower elution volume for more concentrated samples.

Materials required but not supplied

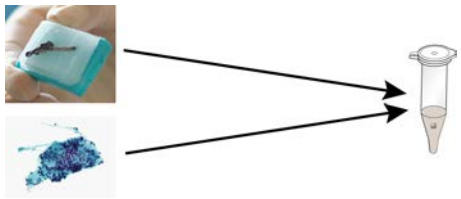
Unless otherwise indicated, all materials are available from Life Technologies. MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Item	Source
Equipment	
Microtome	MLS
Microcentrifuge	MLS
Adjustable micropipettors	MLS
Laboratory mixer (Vortex mixer or equivalent)	MLS
(Optional) Centrifugal vacuum concentrator	MLS
Heating block, 95°C	MLS
2 Heating blocks with lid *or* incubators, 90°C and 55°C	MLS
Wheaton glass 20-slide staining dish with removable rack	Fisher Scientific 08–812
Tubes and accessories	
PureLink™ RNA Mini Columns, 50 columns	A29839
PureLink™ Viral Collection Tubes, 100 tubes	12282100
Nonstick, RNase-free Microfuge Tubes, 1.5 mL, or equivalent	AM12450
Aerosol-resistant pipette tips	MLS
4-way microtube racks	MLS
Feather disposable scalpel #10	Fisher Scientific 50949263
Sterile razor blades	MLS
Reagents	
100% Ethanol	MLS
100% Xylene	MLS
(Optional) Citrisolv Clearing Agent	Fisher Scientific 22-143-975
(Optional) Tris (1 M), pH 8.0	AM9855G
(Optional) EDTA (0.5 M), pH 8.0	AM9260G

Workflow overview

Step 1: Prepare the FFPE samples

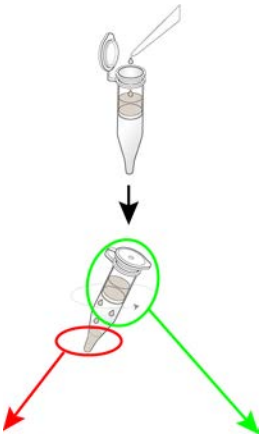
Remove paraffin with xylene, remove excess xylene with ethanol, then dry samples.



Digest the deparaffinized samples with Protease.
Add Protease, incubate at 55°C for 1 hour, then at 90°C for 1 hour.

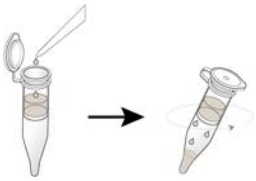
Step 2: Separate RNA from DNA on a PureLink™ column

Add Isolation Additive, then apply the mixture to a PureLink™ column.



Step 3: Recover the RNA from the flow-through

Apply the flow-through after ethanol addition to a PureLink™ column, treat with DNase on the column, then wash and elute the RNA



Step 4: Recover the DNA from the PureLink™ column

Wash and elute the DNA

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- When working with RNA:
  - Wear clean gloves and a clean lab coat.
  - Change gloves whenever you suspect that they are contaminated.
  - Open and close all sample tubes carefully. Avoid splashing or spraying samples.
  - Use a positive-displacement pipettor and RNase-free pipette tips.
  - Clean lab benches and equipment periodically with an RNase decontamination solution, such as RNase*Zap*™ Solution (Cat. no. AM9780).
- Use pre-heated Nuclease-Free Water (Cat. no. AM9938), Low TE buffer (10 mM Tris pH 8.0, 0.1 mM EDTA), or Elution Solution (provided in Cat. no. AM1975) as elution buffer for both RNA and DNA.

We recommend using Nuclease-Free Water if samples are concentrated by vacuum-drying after elution, as salt carryover may interfere with downstream applications.

Before first use of the kit: prepare buffers

- Prepare the Wash Buffers from the concentrates:
  - Add 42 mL of 100% ethanol to Wash 1 Concentrate, then mix well.
  - Add 48 mL of 100% ethanol to Wash 2/3 Concentrate, then mix well.
- *(Optional)* Prepare Low TE buffer according to the following table.

Component	Volume	Final concentration
Tris (1 M), pH 8.0	1 mL	10 mM
EDTA (0.5 M), pH 8.0	20 µL	0.1 mM
Nuclease-Free Water	up to 100 mL	—
Total Low TE buffer	100 mL	—

Store buffers at room temperature.

Before each use of the kit: prepare Digestion Buffer and preheat elution buffer

- Prepare diluted Digestion Buffer for the number of samples required plus 5% overage.

Components	Volume per reaction
Digestion Buffer	25 µL
Nuclease-Free Water	75 µL
Total diluted Digestion Buffer	100 µL

- Preheat elution buffer (see "Procedural guidelines" on page 2) at 95°C.

Step 1: Prepare the FFPE samples

- For curls from FFPE tissue blocks: proceed to "Prepare the curls from FFPE tissue blocks" on page 3.
- For FFPE slide-mounted sections: proceed to "Prepare samples from FFPE slides" on page 4.

Prepare the curls from FFPE tissue blocks

1 Section FFPE tissue blocks

a. Cut sections from FFPE tissue blocks using a microtome.

Note: For miRNA extraction, we recommend using sections of 10 µm or thicker.

b. Collect each section in a 1.5-mL microcentrifuge tube.

2 Remove paraffin from the sections

WARNING! Xylene is a toxic substance. Read the manufacturer's MSDS. Handle it only in a well ventilated area using personal protection equipment. Dispose of xylene waste according to applicable regulations.

a. Preheat a heating block (with lid) or incubator at 50°C.
b. Add 1 mL of 100% xylene or Citrisolv Clearing Agent (or equivalent) to the section, and vortex briefly.
c. Centrifuge briefly to ensure that all the tissue is submerged in xylene.
d. Heat the sample for 3 minutes at 50°C to melt the paraffin.
e. Centrifuge the sample at maximum speed for 2 minutes to pellet the tissue.
   - If the sample does not form a tight pellet, centrifuge again for 2 minutes.
   - If a tight pellet still does not form, proceed with caution to the next step.
f. Remove and discard the xylene.

Note: If the pellet is loose, leave 50–100 µL of xylene in the tube to avoid removing any tissue pieces.

The tissue is usually clear and can be difficult to see.

3 Wash twice with ethanol

WARNING! Discarded ethanol contains trace amounts of xylene and must be handled accordingly.

a. Add 1 mL of 100% ethanol to the tissue pellet and vortex.

The tissue should turn opaque.

b. Centrifuge the sample at maximum speed for 2 minutes.
c. Remove and discard as much ethanol as possible without disturbing the pellet.
d. Perform a second ethanol wash by repeating step 3a through step 3c to ensure complete xylene removal.

IMPORTANT! Omit the second wash when working with small samples as excess washing can result in sample loss.

4 Dry the tissue pellet

Times will vary depending on how much ethanol is present.

Dry the pellet using one of the following methods:

- Use a centrifugal vacuum concentrator with one of the following settings.

Temperature	Time
40–45°C (medium heat)	<20 minutes
37–40°C (low heat)	20–40 minutes

- Air dry at room temperature for 15–45 minutes.

STOPPING POINT *(Optional)* The dried samples can be stored at room temperature up to 72 hours.

5 Digest with Protease

a. Add 100 µL of diluted Digestion Buffer (see "Before each use of the kit: prepare Digestion Buffer and preheat elution buffer" on page 3) and 4 µL of Protease to each sample.
b. Gently flick the tube to mix and to immerse the tissue.

   If the tissue sticks to the sides of the tube, use a pipet tip to push the tissue into the solution or centrifuge briefly to immerse the tissue in the solution.
c. Incubate at 55°C for 1 hour, then centrifuge briefly to collect any condensation droplets.

   Note: If you are using an incubator, use a 4-way microtube rack to allow homogeneous incubation of the samples.
d. Incubate at 90°C for 1 hour, then centrifuge briefly to collect any condensation droplets.

Prepare samples from FFPE slides

1 Remove paraffin from the sections

WARNING! Xylene is a toxic substance. Read the manufacturer's MSDS. Handle it only in a well ventilated area using personal protection equipment. Dispose of xylene waste according to applicable regulations.

a. Submerge the slides in 100% xylene or Citrisolv Clearing Agent (or equivalent) for 5 minutes.
b. Remove the slides, then drain the excess xylene by tilting the slide holder.
c. Submerge the slides in 100% ethanol for 5 minutes.
d. Remove the slides, then drain the excess ethanol by tilting the slide holder.
e. Air dry the slides for 15 minutes.

2 Digest with Protease

a. Add 100 µL of diluted Digestion Buffer to a sterile 1.5-mL tube.
b. Pipet 2–4 µL of diluted Digestion Buffer depending on the tissue size evenly across the FFPE tissue section on the slide to pre-wet the section.

   Note: You can adjust the volume of Digestion Buffer if the tissue is smaller or larger.
c. Scrape the tissue sections in a single direction with a clean razor blade or scalpel, then collect the tissue on the slide into a cohesive mass.
d. Transfer the tissue mass into the tube containing the diluted Digestion Buffer with the scalpel or a pipette tip.
e. Add 4 µL of Protease to each sample.
f. Gently flick the tube to mix and to immerse the tissue.

   If the tissue sticks to the sides of the tube, use a pipette tip to push the tissue into the solution or centrifuge briefly to immerse the tissue in the solution.
g. Incubate at 55°C for 1 hour, then centrifuge briefly to collect any condensation droplets.

   Note: If you are using an incubator, use a 4-way microtube rack to allow homogeneous incubation of the samples.
h. Incubate at 90°C for 1 hour, then centrifuge briefly to collect any condensation droplets.

Step 2: Separate RNA from DNA on a PureLink™ column

1 Pass each sample through a PureLink™ column; save the flow-through and the PureLink™ column

a. Add 120 µL of Isolation Additive to the sample and mix by pipetting up and down.

   The sample will turn slightly cloudy.
b. Add the sample to the PureLink™ column and close the lid.
c. Centrifuge at 10,000 × *g* for 30 seconds.
d. Place the PureLink™ column in a new collection tube and store at room temperature, on ice, or at 4°C, for DNA purification in later steps ("Step 4: Recover the DNA from the PureLink™ column" on page 5).
e. Proceed to RNA purification ("Step 3: Recover the RNA from the flow-through" on page 4) with the flow-through in the tube.

Step 3: Recover the RNA from the flow-through

1 Load the flow-through onto a new PureLink™ column

a. Add 1.25 volumes (usually 275 µL) of 100% ethanol to the flow-through from "Step 2: Separate RNA from DNA on a PureLink™ column" on page 4.
b. Mix well by pipetting up and down and transfer the sample to a new PureLink™ column.
c. Centrifuge at 10,000 × *g* for 30 seconds.
d. Discard the flow-through and re-insert the PureLink™ column in the same tube.

2 Wash the RNA bound to the PureLink™ column

a. Add 600 µL of Wash 1 Buffer to the PureLink™ column.
b. Centrifuge at 10,000 × *g* for 30 seconds.
c. Discard the flow-through and re-insert the PureLink™ column in the same Collection Tube.
d. Centrifuge at 10,000 × *g* for 30 seconds to remove residual fluid.

3 Treat the RNA on the PureLink™ column with DNase

a. Combine the following reagents to prepare the DNase Mix:

Component	Volume per column
DNase	4 µL
10X DNase Buffer	6 µL
Nuclease-Free Water	50 µL
Total volume of DNase Mix	60 µL

b. Add 60 µL of DNase Mix to the center of each PureLink™ column .
c. Incubate at room temperature for 15–30 minutes.

4 Wash the RNA bound to the PureLink™ column

a. Add 600 µL of Wash 1 Buffer to the PureLink™ column.
b. Incubate for 30–60 seconds at room temperature, then centrifuge at 10,000 × *g* for 30 seconds.
c. Discard the flow-through and re-insert the PureLink™ column in the same Collection Tube.
d. Add 500 µL of Wash 2/3 Buffer to the PureLink™ column and centrifuge at 10,000 × *g* for 30 seconds.
e. Discard the flow-through and re-insert the PureLink™ column in the same Collection Tube.
f. Perform a second wash with Wash 2/3 Buffer by repeating step 4d and step 4e.
g. Centrifuge at maximum speed for 2 minutes to remove residual fluid and transfer the PureLink™ column to a new 1.5-mL non-stick RNase-free microfuge tube.

5 Elute the RNA

a. Pre-wet the pipet tip 3 times with pre-heated elution buffer.
b. Add 30–50 µL of pre-heated elution buffer to the center of the PureLink™ column.
c. Incubate at room temperature for 1 minute and centrifuge at maximum speed for 1 minute.
d. *(Optional)* Repeat step 5a to step 5c to perform a second elution.

   A second elution maximizes yield, but results in lower RNA concentration.
e. Discard the PureLink™ column and store the recovered RNA in the Elution Tube at –20°C or –80°C.

Step 4: Recover the DNA from the PureLink™ column

1 Wash the DNA bound to the PureLink™ column

a. Add 600 µL of Wash 1 Buffer to the PureLink™ column set aside from "Step 2: Separate RNA from DNA on a PureLink™ column" on page 4.
b. Centrifuge at 10,000 × *g* for 30 seconds.
c. Discard the flow-through and re-insert the PureLink™ column in the same Collection Tube.
d. Add 500 µL of Wash 2/3 Buffer to the PureLink™ column and centrifuge at 10,000 × *g* for 30 seconds.
e. Discard the flow-through and re-insert the PureLink™ column in the same Collection Tube.

2 Wash the DNA bound to the PureLink™ column

a. Add 500 µL of Wash 2/3 Buffer to the PureLink™ column and centrifuge at 10,000 × *g* for 30 seconds.
b. Discard the flow-through and re-insert the PureLink™ column in the same Collection Tube.
c. Centrifuge at maximum speed for 2 minutes to remove residual fluid and transfer the PureLink™ column to a new 1.5-mL non-stick RNase-free microfuge tube.

3 Elute the DNA

a. Pre-wet the pipet tip 3 times with pre-heated elution buffer.
b. Add 30–50 µL of pre-heated elution buffer to the center of the PureLink™ column.
c. Incubate at room temperature for 1 minute and centrifuge at maximum speed for 1 minute.
d. *(Optional)* Repeat step 3a to step 3c to perform a second elution.

   A second elution maximizes yield, but results in lower DNA concentration.
e. Discard the PureLink™ column and store the recovered DNA in the Elution Tube at –20°C or –80°C.

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Revision history: Revision history of Pub. no. MAN0010642

Revision	Date	Description
D.0	17 June 2016	Correction of the steps recommended to scrap sections.
C.0	13 May 2016	• Update to the recommended spin columns • Optimization of the Protease digestion • Reorganization of the steps
B.0	05 November 2014	Workflow optimization: • Specified optional stopping points • In RNA recovery, removed requirement for Wash 2/3 before DNase treatment • In DNA recovery, reduced the number of washes for samples that are not treated with RNase • Added Elution Buffer and Low TE as elution solution
A.0	24 June 2014	New document

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