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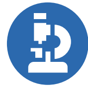
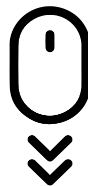
 Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

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- Mindermengenzuschlag
- Trockeneiszuschlag
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Quick-DNA/RNA™ FFPE Kit

DNA & RNA from any sample

Highlights

- Spin-column purification of DNA and total RNA (including small/microRNAs) from FFPE (formalin-fixed paraffin embedded) tissue sections.
- Proteinase K and DNase I enzymes are included for efficient lysis and DNA-free RNA.
- DNA & RNA can be eluted in one eluate or in two separate fractions, ready for Next-Gen Sequencing, RT/qPCR, etc.

Catalog Numbers:
R1009

Scan with your smart-phone camera to view the online protocol/video.

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Product Contents

Quick-DNA/RNA™ FFPE Kit	**R1009** (50 prep)
Deparaffinization Solution	20 ml
Proteinase K[1] (lyophilized) & Storage Buffer	5 mg (x2)
2X Digestion Buffer	5 ml
DNA/RNA Lysis Buffer	50 ml
DNA/RNA Prep Buffer	50 ml
DNA/RNA Wash Buffer[2] (concentrate)	24 ml (x2)
DNase/RNase-Free Water	30 ml
DNase I[3] (lyophilized)	250 U
DNA Digestion Buffer	4 ml
Zymo-Spin™ IICR Columns	100
Collection Tubes	150
Instruction Manual	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add **Proteinase K Storage Buffer** to the lyophilized **Proteinase K**, 5 mg, see Buffer Preparation, page 4. Store frozen aliquots.
2 Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate
3 Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
#E1009-A (250 U), add 275 µl **water**

Specifications

- **Sample Sources –** Up to 25 mg tissue from paraffin block or up to 4 tissue sections (≤ 20 µm thick) with a total surface area of ~ 20 mm^2. Recommended: Use 1-2 sections if performing the protocol for the first time.

- **Compatibility –** Fresh or frozen tissue specimens can also be processed.

- **Size –** DNA and total RNA (≥ 17 nt).

- **Purity –** A_{260}/A_{280} & A_{260}/A_{230} > 1.8. DNA & RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.

- **Binding Capacity – Zymo-Spin™ IICR Column** yield up to 50 µg DNA/RNA.

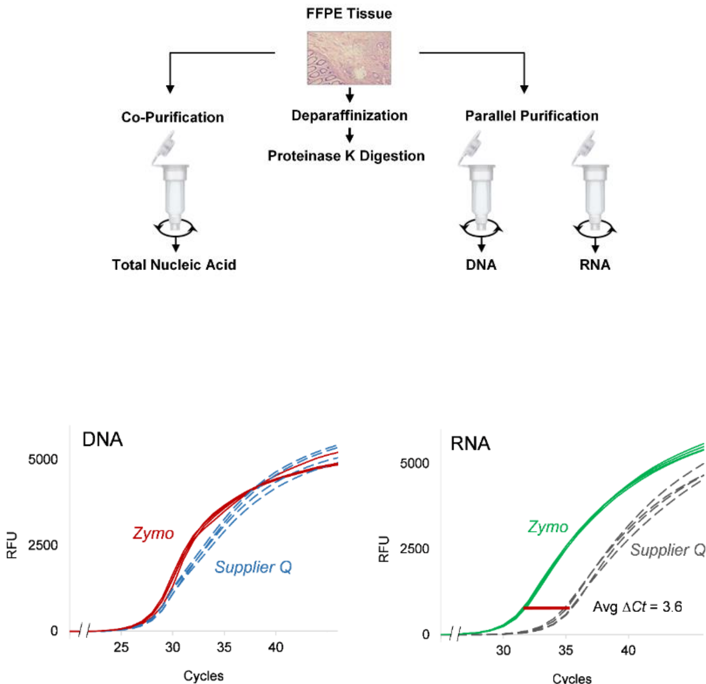
- **Elution Volume –** ≥ 25 µl **DNase/RNase-Free Water.**

- **Equipment Needed** (user provided) **–** Microcentrifuge, vortex, heat block, water bath or incubator.

Product Description

The **Quick-DNA/RNA™ FFPE Kit** provides a simple and reliable method for total nucleic acid isolation (DNA/RNA) in one eluate or DNA and RNA in two separate fractions, from the same formalin-fixed paraffin embedded (FFPE) tissue sample. The unique chemistries of the product have been optimized for maximum recovery of DNA and of both large and small RNA species.

Simply deparaffinize tissues using **Deparaffinization Solution**, digest using **Proteinase K**, heat to reverse chemical crosslinks, and then purify using **Zymo-Spin™ Column** technology. The result is high-quality DNA & total RNA (including small RNAs) that is DNA-free and is ready for RT/qPCR, hybridization, sequencing, etc.



DNA & RNA isolated using the ***Quick*-DNA/RNA™ FFPE Kit** are high quality and consistently outperforms RNA isolated using Supplier Q procedures (Avg Δ Ct = 3.6) as depicted by the RT/qPCR amplification curves (n=4).

Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation, (III) Total Nucleic Acid Purification and (IV) DNA & RNA Purification

(I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate.
- ✓ Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
  #E1009-A (250 U), add 275 µl **water**
  #E1011-A (1500 U), add 1,500 µl **water**
- ✓ Reconstitute lyophilized **Proteinase K** at 20 mg/ml with **Proteinase K Storage Buffer** and mix by vortexing. Use immediately or store frozen aliquots:
  #D3001-2-5 (5 mg), add 0.26 ml **buffer**

(II) Sample Preparation

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

Deparaffinization[1,2]

1. Remove (trim) excess paraffin wax from sample[3] and transfer into a nuclease-free tube (not provided).

2. Add 400 µl of **Deparaffinization Solution** to the sample. Incubate at 55°C for 1 minute. Vortex briefly.

3. Remove **Deparaffinization Solution** from the sample and proceed to Tissue Digestion, below.

Tissue Digestion

1. To the deparaffinized tissue sample (≤ 25 mg), add the following mixture:

DNase/RNase-Free Water	95 µl
2X Digestion Buffer	95 µl
Proteinase K	10 µl

2. Incubate at 55°C for 1 hour (microdissection) or up to 4 hours (tissue block).

3. To de-crosslink the sample, incubate at 94°C for 20 minutes.

4. Proceed to Total Nucleic Acid Purification, page 6 or DNA & RNA Purification, page 7.

1 If using fresh or frozen tissue specimens, proceed directly to Tissue Digestion.
2 Alternatively, xylene may also be used for deparaffinization. See page 8.
3 Up to 25 mg tissue from paraffin block or up to 4 tissue sections (≤ 20 µm thick) with a total surface area of ~20 mm^2. Recommended: Use 1-2 sections if performing the protocol for the first time.

(III) Total Nucleic Acid Purification

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Add 600 µl **DNA/RNA Lysis Buffer** to the tissue and mix thoroughly. Centrifuge at max speed for 1 minute to remove insoluble debris and then transfer the supernatant into a nuclease-free tube (not provided).

2. Add 1 volume ethanol (95-100%) to the supernatant (1:1) and mix well.

   Example: Add 600 µl ethanol to 600 µl supernatant.

3. Then transfer the mixture into a **Zymo-Spin™ IICR Column**[1] in a **Collection Tube** and centrifuge. Discard the flow-through.

   At this point, **DNase I** treatment can be performed, see page 9.

4. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

5. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

6. Add 400 µl **DNA/RNA Wash Buffer** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).

7. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

   Alternatively, for highly concentrated DNA/RNA use ≥ 25 µl elution.

   The eluted DNA/RNA can be used immediately or stored frozen.

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1 To process samples > 700 µl, columns may be reloaded.

(IV) DNA and RNA Purification (in two separate fractions)

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Add 600 µl **DNA/RNA Lysis Buffer** to the tissue and mix thoroughly. Centrifuge at max speed for 1 minute to remove insoluble debris.

2. Transfer the supernatant into a **Zymo-Spin™ IICR Column**[1] into a **Collection Tube** and centrifuge. **<u>Save the flow-through</u>!**

DNA Purification
(DNA is in the column)

3a. Transfer the **Zymo-Spin™ IICR Column**[1] into a new **Collection Tube**.

,

RNA Purification
(RNA is in the flow-through)

3b. Add 1 volume ethanol (95-100%) to the flow-through (1:1) and mix well.

Example: Add 600 µl ethanol to 600 µl flow-through.

Then transfer the mixture into a new **Zymo-Spin™ IICR Column**[1] in a **Collection Tube** and centrifuge[2]. Discard the flow-through.

At this point, **DNase I** treatment (recommended) can be performed (see page 9).

4. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

5. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

6. Add 400 µl **DNA/RNA Wash Buffer** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).

7. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

Alternatively, for highly concentrated DNA/RNA use ≥ 25 µl elution.

The eluted DNA/RNA can be used immediately or stored frozen.

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1 To process samples > 700 µl, columns may be reloaded.

Appendices

Xylene Deparaffinization

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

Slide Tissue Sections Only (rapid deparaffinization)

1. Remove (trim) excess paraffin wax from sample and transfer the sample to a nuclease-free tube (not provided).
2. Add 1 ml xylene (not provided) to the sample. Vortex vigorously for 30 seconds and then centrifuge for 1 minute. Remove and discard the xylene.
3. Wash sample with 1 ml ethanol (95-100%). Vortex vigorously for 30 seconds then centrifuge. Remove and discard ethanol. Repeat this step.
4. Dry the sample using vacuum centrifugation (e.g., SpeedVac) or by heating uncapped tubes at 37° C for up to 40 minutes.
5. Continue to Tissue Digestion, page 5.

Tissue Samples and Slide Tissue Sections (standard deparaffinization)

1. Remove (trim) excess paraffin wax from sample and transfer the sample to a nuclease-free tube (not provided).
2. Add 1 ml xylene (not provided) to the sample. Vortex and incubate at room temperature for 1 hour with gentle rocking. Centrifuge, discard supernatant. Repeat this step.
3. Wash with 1 ml ethanol (100%) for 5 minutes with gentle rocking. Centrifuge and discard supernatant. Repeat this step.
4. Wash with 1 ml ethanol (95%) for 5 minutes with gentle rocking. Centrifuge and discard supernatant. Repeat this step.
5. Wash with 1 ml ethanol (75%) for 5 minutes with gentle rocking. Centrifuge and discard supernatant. Repeat this step.
6. Wash with 1 ml ddH_2O for 5 minutes with gentle rocking. Remove the water from the sample as much as possible.
7. Continue to Tissue Digestion, page 5.

Appendices

DNase I Treatment (in-column)

1. Following RNA binding step (page 7, step 3b), add 400 µl **DNA/RNA Wash Buffer** to the column, centrifuge and discard the flow-through.
2. For each sample to be treated, prepare **DNase I Reaction Mix** in a nuclease-free tube (not provided) and mix by gentle inversion. Then add 80 µl directly into column matrix and incubate at room temperature (20-30°C) for 15 minutes. Proceed with the purification protocol (page 7, step 4).

DNase I Reaction Mix	
DNase I (reconstituted; 1 U/ul)[1]	5 µl
DNA Digestion Buffer	75 µl

Ordering Information

Product Description	Catalog No.	Size
Quick-DNA/RNA™ FFPE Kit	R1009	50 preps.

Individual Kit Components	Catalog No.	Amount
DNA/RNA Lysis Buffer	D7001-1-50 D7001-1-200	50 ml 200 ml
DNA/RNA Prep Buffer	D7010-2-25 D7010-2-50	25 ml 50 ml
DNA/RNA Wash Buffer (concentrate)	D7010-3-12 D7010-3-24	12 ml 24 ml
DNase/RNase-Free Water	W1001-6 W1001-30	6 ml 30 ml
DNase I Set (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
Deparaffinization Solution	D3067-1-20	20 ml
Proteinase K (lyophilized) **& Storage Buffer**	D3001-2-5 D3001-2-20	5 mg 20 mg
2X Digestion Buffer	D3050-1-5 D3050-1-20	5 ml 20 ml
Zymo-Spin™ IICR Columns	C1078-50	50
Collection Tubes	C1001-50	50

Complete Your Workflow

✓ For tough-to-lyse samples, use ZR BashingBead Lysis Tubes:

ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	Plant/animal tissue
0.1 + 0.5 mm beads #S6012	Microbes
0.1 + 2.0 mm beads #S6014	Microbes in tissue/insects

✓ For isolation of DNA/RNA from any sample:

Quick-DNA/RNA Plus kits	
Miniprep Plus #D7003	For cells, tissue, biological liquids
MagBeads #R2130	Automatable (Tecan, Hamilton, Kingfisher, etc.)

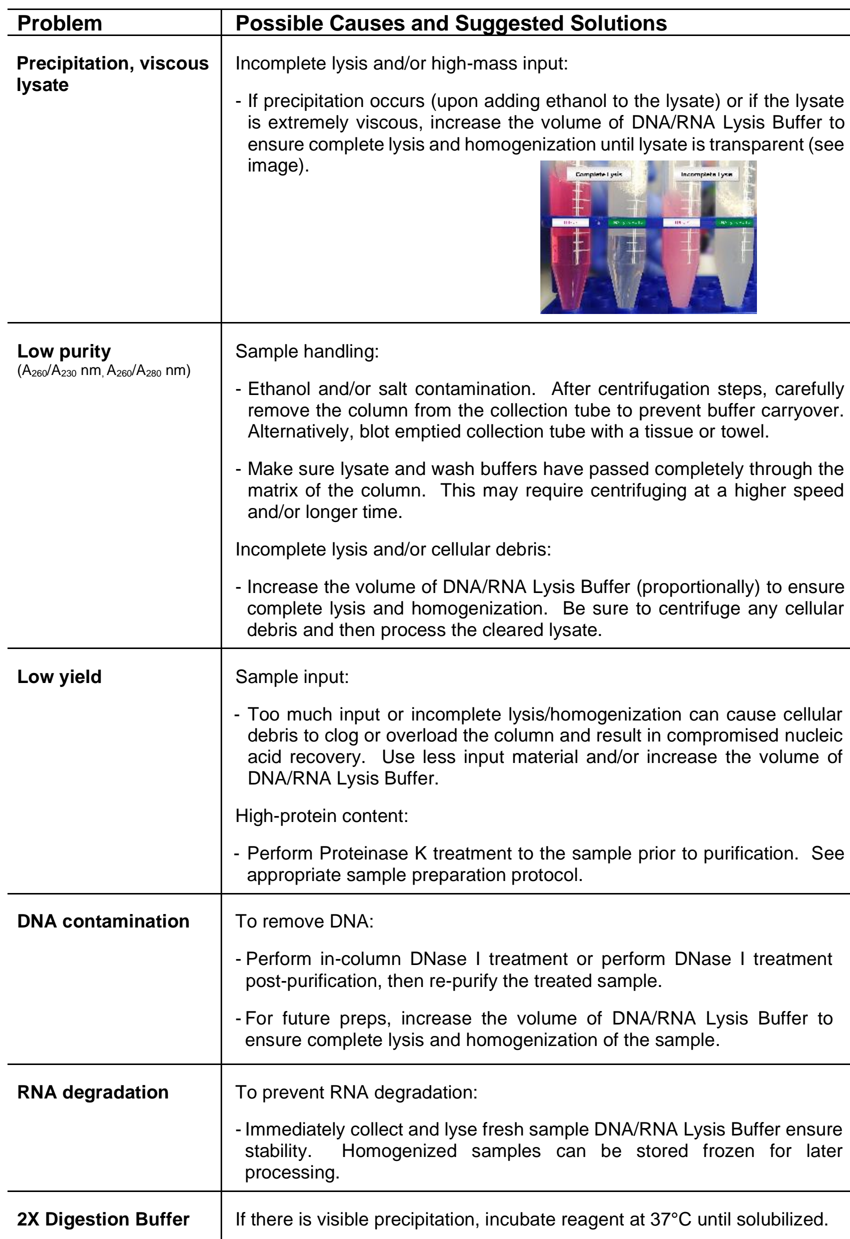
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

RNA Clean & Concentrator kits	
Microprep #R1013-R1014	DNase I Set included
MagBeads #R1082	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For NGS:

Zymo-Seq RiboFree Total RNA Library Prep kit	
#R3000	12 preps
#R3003	96 preps

Troubleshooting Guide



Problem	Possible Causes and Suggested Solutions
Precipitation, viscous lysate	Incomplete lysis and/or high-mass input: - If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of DNA/RNA Lysis Buffer to ensure complete lysis and homogenization until lysate is transparent (see image).
Low purity (A_{260}/A_{230} nm, A_{260}/A_{280} nm)	Sample handling: - Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel. - Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time. Incomplete lysis and/or cellular debris: - Increase the volume of DNA/RNA Lysis Buffer (proportionally) to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate.
Low yield	Sample input: - Too much input or incomplete lysis/homogenization can cause cellular debris to clog or overload the column and result in compromised nucleic acid recovery. Use less input material and/or increase the volume of DNA/RNA Lysis Buffer. High-protein content: - Perform Proteinase K treatment to the sample prior to purification. See appropriate sample preparation protocol.
DNA contamination	To remove DNA: - Perform in-column DNase I treatment or perform DNase I treatment post-purification, then re-purify the treated sample. - For future preps, increase the volume of DNA/RNA Lysis Buffer to ensure complete lysis and homogenization of the sample.
RNA degradation	To prevent RNA degradation: - Immediately collect and lyse fresh sample DNA/RNA Lysis Buffer ensure stability. Homogenized samples can be stored frozen for later processing.
2X Digestion Buffer	If there is visible precipitation, incubate reagent at 37°C until solubilized.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

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