ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-DNA/RNA™ Microprep Plus Kit

Catalog No. **D7005**

Highlights

- **Versatile**: Efficient isolation and separation of DNA and RNA from a wide range of sample sources including cells, tissue (biopsy), blood, and biological fluids.
- **NGS-Ready:** Recover all DNA and RNA (including miRNA) with no sample loss. Nucleic acids are ready for use in any downstream application. *DNase I included.*
- **No Minimum Input**: High sensitivity down to one cell.

Contents

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please call 1-888-882-9682.

For assistance, contact us at tech@zymoresearch.com.

Product Contents

***Quick*-DNA/RNA™ Microprep Plus Kit** (Kit Size)	**D7005** (50 Preps.)	**Storage Temperature**
DNA/RNA Lysis Buffer	50 ml	Room Temp.
DNA/RNA Prep Buffer	50 ml	Room Temp.
DNA/RNA Wash Buffer[1] (concentrate)	2x 24 ml	Room Temp.
DNase/RNase-Free Water	10 ml	Room Temp.
DNase I[2] (lyophilized)	1	-20ºC (reconstituted)
DNA Digestion Buffer	4 ml	Room Temp.
DNA/RNA Shield™ (2X concentrate)	25 ml	Room Temp.
PK Digestion Buffer	5 ml	Room Temp.
Proteinase K[3] (w/ Storage Buffer)	20 mg	-20ºC (reconstituted)
Zymo-Spin™ IC-XM Columns	50	Room Temp.
Zymo-Spin™ IC Columns	50	Room Temp.
Collection Tubes	3x 50	Room Temp.
Instruction Manual	1	-

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

[1] Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate before use.
[2] Prior to use, reconstitute the lyophilized DNase I with 275 µl DNase/RNase-Free Water. Mix by inversion. Store aliquots at -20ºC.
[3] Prior to use, reconstitute the lyophilized **Proteinase K** with 1040 µl **Proteinase K Storage Buffer**. Vortex to dissolve. Store at -20ºC.

Specifications

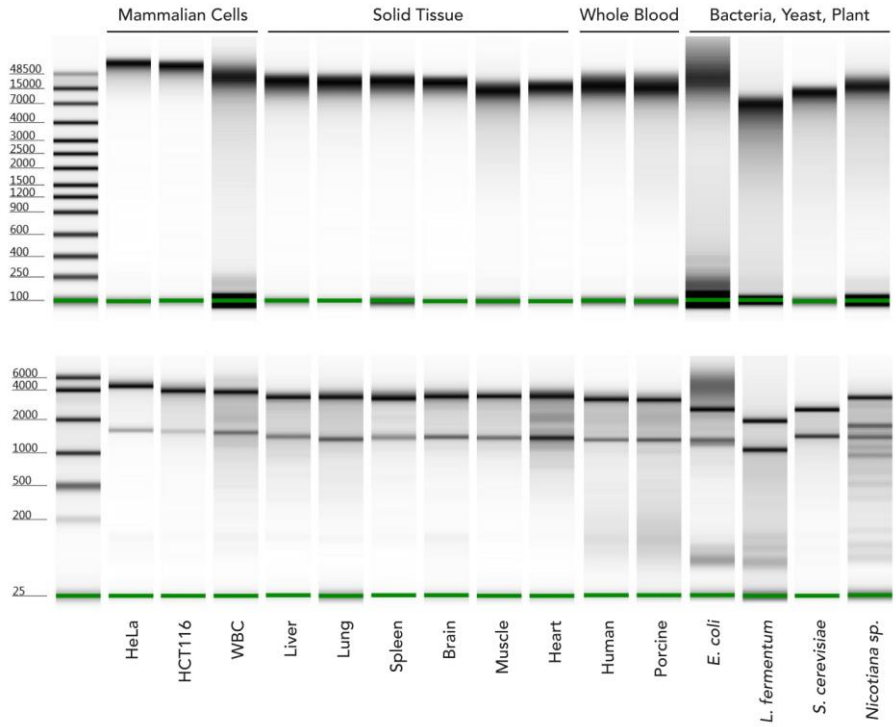
- **Sample Types** – Any cells (animal, blood cells, *etc.*), tissue biopsies (tough-to-lyse, FFPE, *etc.*), microdissections (LCM), needle aspirates, blood, biological fluids, and samples in DNA/RNA Shield™.
- **Sample Preservation** – **DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents and is ideal for safe sample storage and transport at ambient temperatures (page 8).
- **Size** – Capable of recovering genomic DNA ≥40 kb and total RNA ≥17 nt.
- **Purity** – High quality genomic DNA and RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8) are recovered. DNA and RNA is ready for NGS, RT-PCR, microarray, hybridization, *etc.*
- **Yield** – Maximum binding capacity of the provided columns is 5 µg for DNA and 10 µg for RNA. For typical DNA & RNA yields, see page 4.
- **Storage** – DNA and RNA is eluted with DNase/RNase-free water and can be stored at ≤-70 ºC. The addition of RNase inhibitors is highly recommended for prolonged storage.
- **Required Equipment** – Microcentrifuge, vortex, 55°C heat block, water bath or incubator.

Notes:

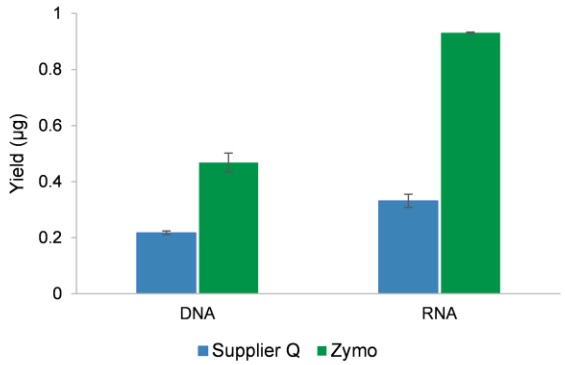
This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

Product Description

The ***Quick*-DNA/RNA™ MicroPrep Plus** kit combines ***Quick*-DNA/RNA™** technology with the addition of **DNA/RNA Shield™**, a unique preservation and lysis technology, and **Proteinase K** to enable easy, reliable, and rapid isolation from any biological sample including cells, solid tissue, and whole blood. The procedure uses *Zymo-Spin*™ column technology that results in high-quality gDNA and total RNA that is ready for any downstream application including reverse transcription, microarray, sequencing.

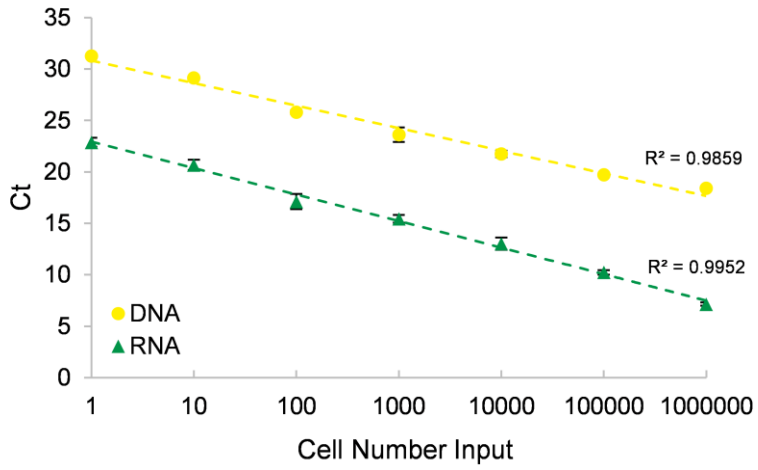


High quality genomic DNA (top) and total RNA (bottom) are isolated from various sample types including mammalian cells, solid tissue, whole blood, bacteria, yeast, and plant using the ***Quick*-DNA/RNA™ Plus** kits (Agilent 2200 TapeStation™).



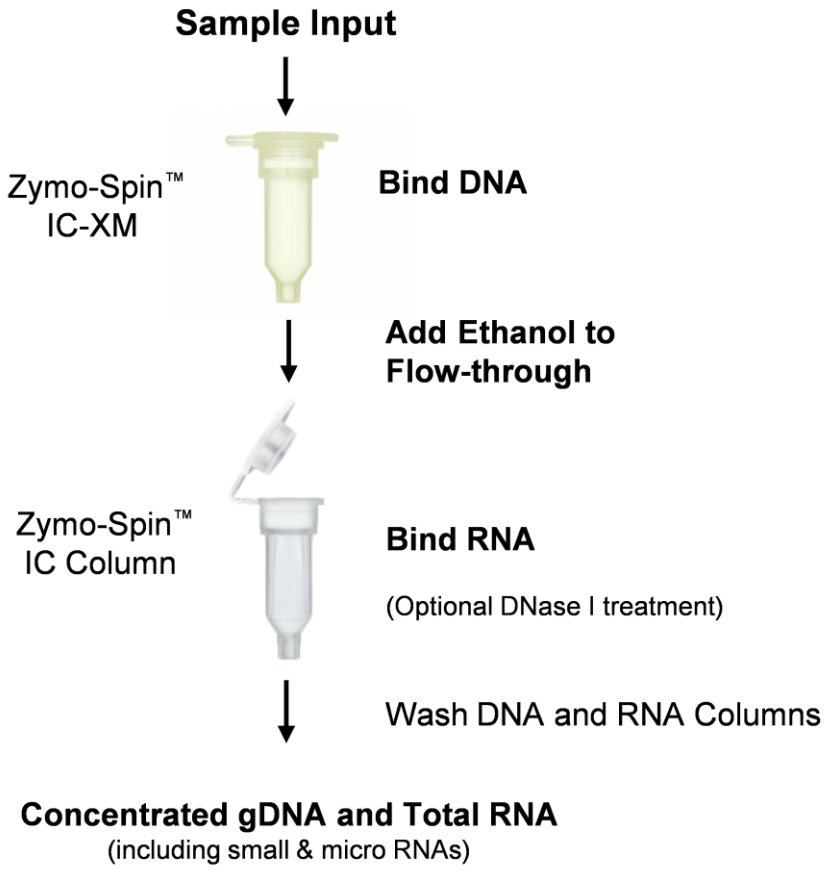
DNA and total RNA recovery is higher using the ***Quick*-DNA/RNA™ Microprep Plus Kit** compared to Supplier Q. Nucleic acids were extracted from 50K HeLa cells (n=2).

For **Technical Assistance**, please contact **Zymo** at 1-888-882-9682 or E-mail tech@zymoresearch.com.



DNA and total RNA were extracted from a decreasing amount of HeLa cells using the ***Quick*-DNA/RNA™ Microprep Plus Kit**. Analysis by RT-qPCR shows high linear recovery of DNA & RNA down to the single cell level (n=2).

Procedure Overview



Purification Guide

Sample Input	Start on
Cells (mammalian, blood cells)	**Page 5**
Tissue (soft, fibrous, lipid, FFPE)	
Whole Blood (biological liquids)	**Page 6**
Tough-to-Lyse (bacteria, yeast, plant)	
Preserved Samples (DNA/RNA Shield™, RNAlater™)	

DNA & RNA Yields and Kit Capacity

Sample Input	Average gDNA Yield	Average RNA Yield	Kit Capacity
Cells	0.4 µg (per 10^5 cells)	1 µg (per 10^5 cells)	Up to 10^6
HeLa	0.6 µg	1.5 µg	
High Yield Tissue[1] (mouse)	≥3 µg (per 1 mg)	≥3 µg (per 1 mg)	Up to 2 mg
Spleen	5-7 µg	3-5 µg	
Liver	1.5-3 µg	4-6 µg	
Low Yield Tissue[1] (mouse)	≤3 µg (per 1 mg)	≤3 µg (per 1 mg)	Up to 5 mg
Brain, Heart	0.5-1.5 µg	0.5-1.5 µg	
Muscle	0.5-1.5 µg	0.5-2 µg	
Lung	1.5-3 µg	1-2 µg	
Intestine	1.5-3 µg	1-3 µg	
Kidney	1.5-3 µg	2-3 µg	
Whole Blood[2]	(per 100 µl)	(per 100 µl)	Up to 200 µl
Porcine	0.5-1 µg	1-2 µg	
Human	0.2-0.5 µg	0.2-1 µg	

Notes:

[1] Yield from tissue samples can vary due to other factors such as organism type, physiological state, and growth conditions.

[2] Yield from blood samples can vary based upon the donor, age, and/or health conditions.

Notes:

The lyophilized **Proteinase K** and **DNase I** are stable as shipped.

[1] Cells in suspension and other liquids may be processed directly by adding 4 volumes of **DNA/RNA Lysis Buffer** and mixing. Proceed to Page 7.

[2] Cell samples homogenized in **DNA/RNA Lysis Buffer** can be stored frozen for processing at a later time.

[3] To prepare 1X solution, mix equal amounts of the supplied 2X concentrate with nuclease-free water (not provided).

FFPE tissue (page 8).

[4] Solid tissue samples should be completely submerged in **DNA/RNA Shield™**, add as needed.

[5] For protein purification, omit step 2 and continue to step 5.

[6] Optimal incubation times may vary with tissue type and homogenization method.

Reagent Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate.
- ✓ Add 275 µl **DNase/RNase-Free Water** per vial to reconstitute the lyophilized **DNase I** at 1 U/µl. Mix by gentle inversion. Store frozen aliquots at -20ºC.
- ✓ Add 1040 µl **Proteinase K Storage Buffer** per vial to reconstitute the lyophilized **Proteinase K** at 20 mg/ml. Vortex to dissolve. Store at -20ºC.

Protocols

The isolation consists of two steps: (I) Sample Preparation & (II) DNA/RNA Purification.

Sample Preparation

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds unless specified. The following procedure should be performed at room temperature (15-30ºC) unless specified.

Cells

Pellet[1] mammalian cells by centrifugation (≤500 *x g* for 1 minute), remove the supernatant and resuspend the cell pellet in **DNA/RNA Lysis Buffer**[2] (see table below). Proceed to Page 7.

Mammalian Cells	Add DNA/RNA Lysis Buffer
≤10^6	400 µl

Solid Tissue & Blood Cells (PBMCs, WBCs)

1. Add **DNA/RNA Shield™** (1X)[3] to a solid tissue sample (see table below). Tissues can be mechanically homogenized for optimal extraction efficiency.

   For blood cells, buffy coat and pelleted PAXgene™ samples, resuspend in **DNA/RNA Shield™** (1X).

Animal Tissue	Blood Cells	Add DNA/RNA Shield™ (1X)
≤5 mg	≤0.5 ml blood	≥300 µl[4]

2. For every 300 µl of sample, add 30 µl **PK Digestion Buffer** and 15 µl **Proteinase K**[5].

3. Mix and then incubate at 55ºC until tissue dissolves or up to 5 hours.[6]

Sample	Incubation Time
Non-homogenized	2-5 hours
Homogenized tissue	30 minutes
Blood cells (or PAXgene™ pellet)	30 minutes

Sample Preparation (continued)

4. After incubation, vortex sample and then centrifuge at max speed for 2 minutes to pellet debris. Transfer the aqueous supernatant into an RNase-free tube (not provided).

5. Add an equal volume of **DNA/RNA Lysis Buffer** and mix well. Proceed to Page 7.

Whole Blood (Mammalian)

1. Add 200 µl[1] **DNA/RNA Shield**™ (2X concentrate) directly to each 200 µl of fresh/frozen blood sample and mix thoroughly.

2. For every 400 µl of reagent/blood mixture, add 8 µl **Proteinase K**[2] and mix thoroughly. Incubate at room temperature (20-30ºC) for 30 minutes.

3. Add an equal volume of isopropanol and mix by vortex. Proceed to Page 7.

Tough-to-Lyse Samples (Bacterial, Yeast, Plant, *etc.*)

Tough-to-lyse samples (including gram-positive bacteria) should be mechanically homogenized (*i.e.* ZR BashingBead™ Lysis Tubes*) directly in **DNA/RNA Shield**™ (1X; mix equal amounts of the supplied 2X concentrate with nuclease-free water (not supplied)). Centrifuge and transfer the supernatant into an RNase-free tube. Add an equal volume of **DNA/RNA Lysis Buffer** and mix well. Proceed to Page 7.

Bacterial[3]	Yeast	Plant/Seed	Add DNA/RNA Shield™ (1X)
≤2x10^8	≤2x10^7	≤20 mg	≥800 µl

For the removal of PCR (RT) inhibitors from fecal, soil, plant, use the OneStep™ PCR Inhibitor Removal Kit (D6030).

Liquids

Add 3 volumes of **DNA/RNA Lysis Buffer** for every volume of sample (*e.g.* 300 µl buffer to 100 µl sample). Proceed to Page 7.

Samples Preserved in DNA/RNA Shield™

Bring samples homogenized and stored in **DNA/RNA Shield**™ (1X) to room temperature (20-30ºC). Add 1 volume of **DNA/RNA Lysis Buffer** (1:1) and mix well. Proceed to Page 7.

Samples in RNA*later*™

To process cells or liquids in RNA*later*™ (without reagent removal): Add 1 volume of RNase-free water or PBS to the sample (1:1). Then add 4 volumes **DNA/RNA Lysis Buffer** (4:1) and mix. Proceed to Page 7.

Note: Alternatively, remove the RNAlater™, then proceed with Sample Preparation.

Notes:

[1] Up to 200 µl can be processed without having to reload the spin column.

[2] For protein purification, omit step 2 and continue to step 3.

**For bacterial, fungal, fecal, and soil samples, use the 0.5 mm beads (S6002).*

**For plant/seed, solid tissues, and insect samples, use the 2.0 mm beads (S6003).*

[3] Some Gram-negative bacteria (*e.g. E. coli*) may not require the **BashingBead**™ system and can be lysed directly in **DNA/RNA Shield**™.

Sample preservation with DNA/RNA Shield™ (page 8).

Notes:

[1] To process samples >700 µl, **Zymo-Spin**™ columns may be reloaded.

[3] Alternatively, to isolate RNAs ≥200 nt, add ½ volume ethanol (95-100%) to the sample flow-through.

[4] Save the flow-through for protein purification (page 8).

[5] At this point, RNA samples can be in-column DNase I treated (page 8).

Purification Protocol

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds unless specified. The following procedure should be performed at room temperature (15-30ºC) unless specified.

1. Transfer the sample into a **Zymo-Spin**™ **IC-XM Column** in a **Collection Tube** and centrifuge.

   Save the flow-through.

   For whole blood only:

   Discard the flow-through from above. Transfer the filter into a clean microcentrifuge tube (not provided). Add 200 µl **DNA/RNA Lysis Buffer** directly to the filter matrix, let stand 5 minutes and then centrifuge. **Save the flow-through.**

   Save the flow-through for RNA and the column for DNA purification! Proceed below.

DNA Purification (DNA is bound to the column)	**RNA Purification** (RNA is in the flow-through)
2. Transfer the **Zymo-Spin**™ **IC-XM Column** into a new **Collection Tube**.	2. Add an equal volume[3] of ethanol (95-100%) to the flow-through and mix well. Then transfer the sample into a **Zymo-Spin**™ **IC Column**[1] in a **Collection Tube** and centrifuge. Discard the flow-through.[4,5]

3. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
4. Add 700 µl **DNA/RNA Wash Buffer** and centrifuge. Discard the flow-through.
5. Add 400 µl **DNA/RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Carefully transfer the column into a clean microcentrifuge tube.
6. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix, let stand for 5 minutes, and then centrifuge to elute DNA and RNA from the respective column.

   Alternatively, for highly concentrated DNA and RNA, use ≥6 µl elution.

   The eluted DNA & RNA can be used immediately or stored at ≤-70ºC.

Appendix A: Sample Preservation in DNA/RNA Shield™

DNA/RNA Shield™ effectively lyses cells, inactivates nucleases and infectious agents and is ideal for sample storage/transport at ambient temperatures prior to nucleic acid purification.

Liquid samples: Mix an equal volume DNA/RNA Shield™ (2X concentrate) and sample.
Solid samples: Submerge sample (not to exceed 10% (v/v or w/v) in DNA/RNA Shield (1X).

Mix well/homogenize sample prior to storage.

Samples in DNA/RNA Shield™ can be stored at ambient temperature (4-25ºC) for a month, 3 days at 37ºC or long term (>1 year) at -20ºC or below.

Appendix B: *In-Column* DNase I Treatment

The DNase I digestion procedure can be performed using **DNase I Set** (E1010).[1]
All centrifugation steps should be performed at 10,000 –16,000 *x g* for 30 seconds unless specified.

1. Wash the column with 400 µl **DNA/RNA Wash Buffer** and centrifuge. Discard the flow-through.
2. Add 40 µl **DNase I Reaction Mix** (below) directly to the column matrix.

DNase I	5 µl (1 U/µl)*
DNA Digestion Buffer	35 µl

3. Incubate the column at room temperature (20-30ºC) for 15 minutes.
Continue with RNA Purification: Page 7, Step 3.

Appendix C: Acetone Precipitation of Proteins

1. Add 4 volumes of cold acetone (-20 ºC) to flow-through with ethanol obtained after the RNA binding in *RNA Purification.*
2. Incubate samples for 30 minutes on ice.
3. Centrifuge at top speed for 10 minutes. Discard the supernatant. Keep the pellet!
4. Add 400 µl ethanol (95-100%) to the protein pellet. Centrifuge at top speed for 1 minute. Discard the supernatant.
5. Air-dry protein pellet for 10 minutes at room temperature.
6. Resuspend and vortex the pellet in a buffer appropriate for downstream application. For example: SDS-PAGE sample loading buffer.

Appendix D: DNA/RNA Purification from FFPE Tissue: Deparaffinization

1. Remove (trim) as much excess paraffin from the sample as possible.
2. Transfer sample to a microcentrifuge tube (not provided).
3. Add 1 ml xylene and mix well. Then centrifuge for 1 minute and remove xylene.
4. Add 1 ml ethanol (95-100%) and mix well. Then centrifuge for 1 minute and remove ethanol. Repeat this step.
5. Dry samples by vacuum centrifugation (Speed-Vac) or by incubating uncapped tubes at ≤37 °C. It may take up to 40 minutes for a sample to air dry.
6. To purify DNA/RNA, follow Sample Preparation for tissue (Page 5) and DNA/RNA Purification (Page 7).

Notes:

[1] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

** Unit definition - one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/ml of reaction mixture at 25°C.*

Troubleshooting Guide:

For **Technical Assistance**, please contact 1-888-882-9682 or E-mail tech@zymoresearch.com.

Problem	Possible Causes and Suggested Solutions
Sample Degradation	
Sample Preservation	• **Tissue Stabilization:** Immediately submerge tissue samples in **DNA/RNA Shield™** to ensure nucleic acid stability. Various tissues are rich in RNases that can digest RNA quickly unless proper freezing or stabilization is used. • **Blood Preservation:** Using frozen whole blood (EDTA, citrate) samples can cause cell damage from membrane shock and shearing, resulting in degradation. For best results, store fresh whole blood in DNA/RNA Shield™ (2X concentrate). Alternatively, add the DNA/RNA Shield to frozen blood samples prior to thawing
Quality Control	• **Establish Controls:** Check performance of kit (buffers and columns) with a well defined sample input of known quality and concentration to eliminate artifacts originating from kit to kit variation.
Low Yield	
Sample Input	• **Sample Overloading:** For "high yield" samples, if the lysate is extremely viscous or did not pass through the column, use less input material. Too much input can cause cellular debris to overload the column and result in compromised recovery. • **Low Nucleic Acids Content:** For "low yield" tissue (e.g., muscle), using larger inputs will increase yields (≤ 5 mg). Refer to the "DNA & RNA Yields" table (page 4).
Proteinase K	• **Incomplete Lysis/Digestion:** Proteinase K incubation times may be extended depending on the type of sample (e.g., fibrous tissue).
Elution	• **Incomplete Elution:** Reload the eluate onto the column and centrifuge again. Alternatively, heat the nuclease-free water to 95ºC before use.
Formation of white precipitate	• **Sample Overloading:** Precipitation of nucleic acids occurs due to inefficient lysis. Decrease the starting sample input and/or increase the volume of lysis buffer used.
Residual DNA	
DNase	• **DNase Treatment:** Perform *in-column* DNase I treatment (page 8) to remove DNA from the RNA fraction. DNase I treatment can also be performed on the RNA elution after purification ("in-tube") and cleaned up again to remove DNase enzyme.
Sample Input	• **Sample Overloading:** For "high-yield" samples, do not overload the Zymo-Spin™ IC-XM (titrate the input if necessary).
Low Purity ($A_{260/230\ nm}$)	
Sample Handling	• **Salts & Contaminants:** There may be ethanol and/or salt contamination. Carefully remove the column from the collection tube so that there is no liquid contact. Blot emptied collection tubes with a tissue or towel to minimize liquid retention. Additional wash steps may also be performed to help remove residual contaminants. • **Column Clogging:** Make sure lysate has passed completely through matrix in the column before proceeding to wash steps. This may require centrifuging at a higher speed and/or longer time. More lysis buffer and/or ethanol can be added to further dilute the sample to help pass through the column. • **Binding:** Make sure to use only the cleared supernatant for binding. Any solid debris stemming from tissue digestion or similar remaining on the column can affect final sample quality.

Ordering Information

Product Description	Kit Size	Catalog No.
***Quick*-DNA/RNA™ Microprep Plus Kit**	50 Preps.	D7005
***Quick*-DNA/RNA™ Miniprep Kit**	50 Preps.	D7001
***Quick*-DNA/RNA™ Miniprep Plus Kit**	50 Preps.	D7003

For Individual Sale	Amount	Catalog No.
DNA/RNA Lysis Buffer	50 ml	D7001-1-50
DNA/RNA Prep Buffer	10 ml 25 ml 50 ml	D7010-2-10 D7010-2-25 D7010-2-50
DNA/RNA Wash Buffer (concentrate)	6 ml 12 ml 24 ml	D7010-3-6 D7010-3-12 D7010-3-24
DNase/RNase-Free Water	1 ml 4 ml 6 ml 10 ml	W1001-1 W1001-4 W1001-6 W1001-10
DNase I Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	1 set	E1010
DNA/RNA Shield™ (2X concentrate)	25 ml 125 ml	R1200-25 R1200-125
PK Digestion Buffer	5 ml 20 ml	R1200-1-5 R1200-1-20
Proteinase K (lyophilized) supplied with Proteinase K Storage Buffer	5 mg set 20 mg set	D3001-2-5 D3001-2-20
Zymo-Spin™ IC-XM Columns	50	C1103-50
Zymo-Spin™ IC Columns	50 250	C1004-50 C1004-250
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000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