ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZymoBIOMICS™ DNA/RNA Mini Kit

Catalog No. **R2002**

Highlights

- Rapid, robust, and simple purification of high quality, inhibitor-free DNA and total RNA (including small/micro RNAs) from any sample including feces, soil, plant, water, biofilms, swabs, saliva, body fluids, *etc*.
- **ZymoBIOMICS™** innovative lysis system enables efficient and unbiased lysis of microbes including gram positive/negative bacteria, fungus, protozoans, algae, viruses, *etc*.
- High quality DNA and *DNA-free* RNA is ready for use in any downstream application. *DNase I included.*

Contents

ZYMO RESEARCH CORP.

Phone: (949) 679-1190 ▪ Toll Free: (888) 882-9682 ▪ Fax: (949) 266-9452 ▪ info@zymoresearch.com ▪ www.zymoresearch.com

Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please contact us.

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 the highest performance and reliability.

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

Product Contents

ZymoBIOMICS™ DNA/RNA Mini Kit (Kit Size)	**R2002** (50 Preps.)	**Storage Temperature**
DNA/RNA Shield™ - Lysis Tube (Microbe)	50	Room Temp.
DNA/RNA Lysis Buffer	50 ml	Room Temp.
DNA/RNA Prep Buffer	50 ml	Room Temp.
DNA/RNA Wash Buffer[1] (concentrate)	24 ml	Room Temp.
DNase/RNase-Free Water	2x 30 ml	Room Temp.
DNase I[2] (lyophilized)	1	Room Temp.
DNA Digestion Buffer	4 ml	Room Temp.
Zymo-Spin™ IV-HRC Spin Filters (green tops)	100	Room Temp.
Spin-Away™ Filters	50	Room Temp.
Zymo-Spin™ IIICG Columns	50	Room Temp.
Collection Tubes	300	Room Temp.
Instruction Manual	1	

Storage Temperature - Store all kit components (*i.e.,* buffers, columns) at room temperature.

[1] Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate.

[2] Prior to use, reconstitute the lyophilized **DNase I** with 275 µl **DNase/RNase-Free Water**. Mix by gentle inversion. Store aliquots at -20ºC.

Specifications

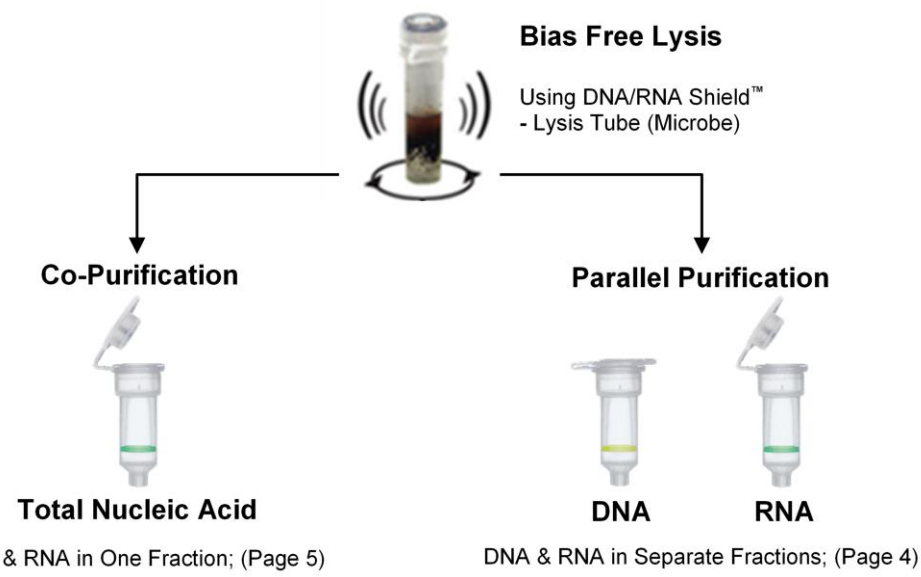
- **Sample Sources** – Bacterial, fungal, protozoan, algae, viral, mitochondrial, and host DNA and RNA is efficiently isolated from ≤ 200 mg of mammalian feces, ≤ 250 mg soil, ≤ 200 mg plant/seed, 50-100 mg (wet weight) fungal bacterial cells[1], biofilms, and water.
- **Bead beating system** – **ZymoBIOMICS™** innovative lysis system ensures complete homogenization of the microbial cell walls and accurate microbial analysis, free of bias.
- **Sample Preservation** – **DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents and is ideal for safe sample storage and transport at ambient temperatures.
- **Size Limits** – Capable of recovering DNA and total RNA ≥17 nucleotides.
- **Purity** – High quality DNA and RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8) are recovered.
- **Recovery** – The DNA/RNA binding capacity of the **Zymo-Spin™ IIICG Column** is ~100 µg.
- **Storage** – DNA and RNA eluted with **DNase/RNase-Free Water** can be stored at ≤70ºC. The addition of RNase inhibitors in highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge, vortex, cell disrupter (recommended).

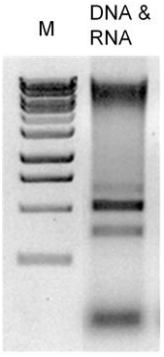
[1] This equates to approximately 10^9 bacterial cells, 10^8 yeast cells, and 10^7 mammalian cells.

™ Trademarks of Zymo Research Corporation. This product is for research use only and should be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow safety guidelines and rules enacted by your research institution or facility. Disruptor Genie® is a registered trademark of Scientific Industries, Inc. and FastPrep® is a registered trademark of Qbiogene, Inc.

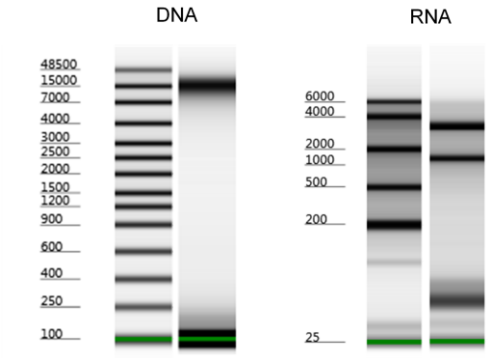
Product Description

The **ZymoBIOMICS™ DNA/RNA Mini Kit** is designed for purifying DNA and RNA from a wide array of sample inputs (*e.g.* feces, soil, plant, water, and biofilms) that is ready for microbiome or metagenome analyses. The ZymoBIOMICS™ innovative lysis system eliminates bias associated with unequal lysis efficiencies of different organisms (*e.g.* gram negative/positive bacteria, fungus, protozoans, and algae). The provided **DNA/RNA Shield™** preserves nucleic acids at ambient temperatures, providing an unbiased molecular snapshot of the sample. The procedure uses *Zymo-Spin®* column technology that results in high-quality DNA and total RNA *(including small RNAs 17-200 nt)* that is free of PCR inhibitors (*e.g.* polyphenols, humic acids, and fulvic acids) and is ready for RT-PCR, arrays, sequencing, *etc*.





Human stool total nucleic acid (DNA & RNA) isolated with the **ZymoBIOMICS™ DNA/RNA Mini Kit** is high quality. Elutions were analyzed in a 1% TAE/agarose/EtBr gel. The size marker "M" is a 1 kb ladder (Zymo Research).



Human stool genomic DNA and total RNA isolated with the **ZymoBIOMICS™ DNA/RNA Mini Kit** is highly intact. Quality assessed by Agilent 2200 TapeStation.

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

The lyophilized **DNase I** is stable as shipped.

Reagent Preparation

- ✓ Before starting, 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.

Protocols

The isolation consists of two steps: (I) Sample Preparation & (II) Parallel Purification or Co-Purification.

Sample Preparation

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) unless specified.

1. Add sample to a **DNA/RNA Shield™ - Lysis Tube (Microbe)**. Cap tightly to assure no leakage during bead beating.

Sample Type	Maximum Input
Feces	200 mg
Soil	250 mg
Plant/Seed	200 mg
Liquid Samples and Water[1]	250 µl
Cells (Suspended in DNA/RNA Shield™ or isotonic buffer, *e.g.* PBS)	50-100 mg (wet weight) (10^9 bacterial, 10^8 yeast cells, 10^7 mammalian cells)

2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for ≥ 5 minutes.

   Processing time will vary based on sample input and bead beater. Times may be as little as 5 minutes when using high-speed cell disrupters (FastPrep®-24) or as long as 20 minutes when using lower speeds (e.g. Disruptor Genie®).

3. Centrifuge the **DNA/RNA Shield™ - Lysis Tube (Microbe)** in a microcentrifuge for 1 minute.
4. Transfer up to 400 µl supernatant to a new microcentrifuge tube (not provided).
5. Add 1 volume of **DNA/RNA Lysis Buffer** to the sample and mix well.

   Proceed to Parallel Purification (page 4) for DNA & RNA in separate fractions or Co-Purification (page 5) for DNA & RNA in one fraction.

Notes:

[1] For water samples, filter using desired filter (not provided). Cut the filter into small pieces and place into DNA/RNA Shield™ - Lysis Tube (Microbe).

DNA & RNA Parallel Purification

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) unless specified.

1. Transfer the sample into a **Spin-Away™ Filter** (**yellow**) in a **Collection Tube** and 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 **Spin-Away™ Filter** (**yellow**) into a new **Collection Tube**.	2. Add 1 volume ethanol (95-100%) to the flow-through and mix well. Then transfer the sample into a **Zymo-Spin™ IIICG Column**[1] (**green**) in a **Collection Tube** and centrifuge. Discard the flow-through.[2]

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** to the column and centrifuge. Discard the flow-through.
5. Add 200 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 100 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 & RNA use ≥50 µl elution.

7. Preparing **Zymo-Spin™ IV-HRC Spin Filter** (green tops)

   a. Snap off the base of the filter and place into a Collection Tube. Centrifuge at 8,000 *x g* for 3 minutes. Discard the flow-through.

   b. Remove the cap and add 400 µl **DNase/RNase-Free Water** to the filter. Loosely cap the filter and centrifuge at 8,000 *x g* for 2 minutes.

8. Transfer the eluted DNA & RNA (Step 6) into a prepared **Zymo-Spin™ IV-HRC Spin Filter** in a new microcentrifuge tube and centrifuge at 8,000 *x g* for 1 minute.

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

Notes:

[1] To process samples >800 µl, **Zymo-Spin™** columns may be reloaded.

[2] At this point, RNA samples can be in-column DNase I treated (page 6).

Notes:

[1] To process samples >800 µl, **Zymo-Spin™** columns may be reloaded.

DNA & RNA Co-Purification

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) unless specified.

1. Add 1 volume ethanol (95-100%) to the sample and mix well.
2. Transfer the mixture into a **Spin-Away™ Filter**[1] (**yellow**) in a **Collection Tube** and centrifuge. Discard the flow-through.
3. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
4. Add 400 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Transfer the column carefully into a new microcentrifuge tube (not provided).
5. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix, let stand for 5 minutes, and then centrifuge to elute.
6. Add 2 volumes of **DNA/RNA Lysis Buffer** to the sample and mix.
7. Add an equal volume of ethanol (95-100%) and mix.
8. Transfer the sample into a **Zymo-Spin™ IIICG Column** (**green**) in a **Collection Tube** and centrifuge. Discard the flow-through.
9. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
10. Add 700 µl **DNA/RNA Wash Buffer** and centrifuge. Discard the flow-through.
11. Add 400 **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.
12. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix, let stand for 5 minutes, and then centrifuge to elute DNA & RNA into one fraction.

    Alternatively, for highly concentrated DNA/RNA use ≥50 µl elution.

13. Preparing **Zymo-Spin™ IV-HRC Spin Filter** (green tops)

    a. Snap off the base of the filter and place into a Collection Tube. Centrifuge at 8,000 *x g* for 3 minutes. Discard the flow-through.

    b. Remove the cap and add 400 µl **DNase/RNase-Free Water** to the filter. Loosely cap the filter and centrifuge at 8,000 *x g* for 2 minutes.

14. Transfer the eluted DNA/RNA (Step 12) into a prepared **Zymo-Spin™ IV-HRC Spin Filter** in a new microcentrifuge tube and centrifuge at 8,000 *x g* for 1 minute.

    The filtered DNA/RNA can be used immediately or stored at ≤-70ºC.

Appendices

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 80 µl **DNase I Reaction Mix** (below) directly to the column matrix.

DNase I	5 µl (1 U/µl)*
DNA Digestion Buffer	75 µl

3. Incubate the column at room temperature (20-30ºC) for 15 minutes.
Continue with RNA Purification: Page 4, Step 3.

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.*

Ordering Information

Product Description	Catalog No.	Kit Size
ZymoBIOMICS™ DNA/RNA Mini Kit	R2002	50 Preps.

For Individual Sale	Catalog No.	Amount
DNA/RNA Shield™ - Lysis Tube (Microbe)	R1100-1-B15	50
DNA/RNA Lysis Buffer	D7001-1-50	50 ml
DNA/RNA Prep Buffer	D7010-2-10 D7010-2-25 D7010-2-50	10 ml 25 ml 50 ml
DNA/RNA Wash Buffer (concentrate)	D7010-3-6 D7010-3-12 D7010-3-24	6 ml 12 ml 24 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10 W1001-30	1 ml 4 ml 6 ml 10 ml 30 ml
DNase I Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set
Zymo-Spin™ IV-HRC Spin Filters (green tops)	C1010-50	50
Spin-Away™ Filters	C1006-50-F	50
Zymo-Spin™ IIICG Columns	C1006-50-G	50
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000
DNA/RNA Shield™ - Fecal Collection Tube	R1100-9-T	10 tubes
DNA/RNA Shield™ - Collection Tube	R1100-1-T	50
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml

ZYMO RESEARCH

The Beauty of Science is to Make Things Simple