Nov 30, 2025 Version 2

ZymoBIOMICS DNA/RNA Miniprep Kit - Modified Protocol Marine Invertebrate Tissues - ViDaB Lab V.2

DOI
https://dx.doi.org/10.17504/protocols.io.6qpvrw41zlmk/v2

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Protocol Citation: Alex Veglia, Joyah Watkins 2025. ZymoBIOMICS DNA/RNA Miniprep Kit - Modified Protocol Marine Invertebrate Tissues - ViDaB Lab . **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvrw41zlmk/v2Version created by **Alex Veglia**

Protocol status: Working
We use this protocol and it's working

Created: November 29, 2025

Last Modified: November 30, 2025

Protocol Integer ID: 233799

Keywords: rna miniprep kit, zymobiomics dna, zymobiomics innovative lysis system, protocol for nucleic acid extraction, nucleic acid extraction, unbiased lysis of microbe, marine invertebrate tissue, microbe, vidab lab, negative bacteria, zymobiomic, viruses from any sample, innovative lysis system, fungi, biofilm, swab, modified protocol marine invertebrate tissue, sponge tissue, nucleic acid, purity of nucleic acid, dna

Abstract

This is a modified manufacturers protocol that was altered to incease yield and purity of nucleic acids from coral and sponge tissues. From the manufacturer: "ZymoBIOMICS innovative lysis system enables efficient and unbiased lysis of microbes including gram positive/negative bacteria, fungi, protozoans, and viruses from any sample including feces, soil, plant, water, biofilms, swabs, saliva, body fluids, etc."

Materials

ZymoKit Materials
- DNA/RNA Lysis Buffer
- Spin-Away Filters (yellow, green, and white)
- Lo bind Collection Tubes
- DNA/RNA Prep Buffer
- HRC Prep Solution
- ZymoBIOMICS DNase/RNase- Free Water
- DNase I
- DNA Digestion Buffer (For DNase I reaction mix)
- Proteinase K Digestion Buffer
- Proteinase K (in freezer)

Non-Kit Materials
- nuclease free tubes (1.5 - 2 mL)
- 100% ethanol

Before start

Upon opening a new Zymo kit, perform the following:

1. Add 96 mL 100% ethanol to the 24 ml DNA/RNA Wash Buffer concentrate. With a sharpie pen, check the white box on the bottle to indicate ethanol has been added to reagent.

2. [WORKING WITH RNA]: Reconstitute DNase 1 with ZymoBIOMICS DNase/RNase-Free Water, mix by gently inversion and store frozen aliquots.

Sample Preparation

1 UV 3 sets of 1.5 ml tubes in the dead air box (pour tubes out of the bag in the dead air box, be careful not to touch inside of tube lids etc) – 42 tubes (3×14)

2 UV 2 sets of 1.5 ml LoBind tubes in the dead air box (at the same time or later)

3 Turn on the heat block- set it to 50°C OR incubator to 37°C

4 Once thawed, centrifuge at 8,000 xG for 30 seconds. Make sure the centrifuge is set to xg and not rcf for this protocol

5 Remove 400 µL of supernatant from the Zymo tube. Place supernatant in a pre-labeled and pre-UV'ed 1.5ml tube.
NOTE: Place tubes with remaining tissue in their labeled box in the -80 freezer

6 Proceed to *Enzyme Lysis Steps*

Enzyme Lysis Steps

7 Add 40 µL of Proteinase K Digestion Buffer to the sample
Buffer is okay to leave at room temperature. To make 1 mL buffer:
- 20 µL of 5M NaCl
- 10 µL of 1M Tris pH 9.0
- 2 µL of 0.5M EDTA
- 50 µL of 10% SDS
- 918 µL RNA/DNAse Free Water

8 Add 20 µL of Proteinase K

9 Incubate for 2.5 hours at 50°C.

10 Proceed to *DNA and RNA Purification* after incubation.

DNA and RNA Purification

11 Add 460 µL **DNA/RNA Lysis Buffer** and vortex for 2-3 seconds (or mix well).

11.1 Transfer 460 µL of sample into a **Spin-Away Filter** (yellow) in a **2 mL centrifuge tube**.
DO NOT DISCARD THE OTHER HALF OF SAMPLE!!!

11.2 Centrifuge at 16,000 x G for 1 minute. **Save flow through in a 2 mL tube.**

11.3 Transfer the Spin-Away Filter (yellow) into a new Collection Tube.

11.4 Transfer the remaining 460 µL of sample into the same yellow Spin-Away Filter. Ensure the yellow filter is on the same 2 mL centrifuge tube containing the flow through.

11.5 Centrifuge at 16,000 x G for 1 minute. **Save flow through in a 2 mL tube.**

11.6 Transfer the Spin-Away Filter (yellow) into a new Collection Tube.

11.7 [RNA PURIFICATION]: Add an equal volume (e.g., 920 µL) of ethanol (95 - 100%) to the flow-through in a 2 mL tube (1:1) and mix well. Then transfer the sample into a **Zymo-Spin IILCG Column** (green) in a **Collection Tube** and centrifuge (16,000 xG for 1 minute). Discard the flow through.

DNase I Treatment (in-column) - RNA Sample(s) ONLY

12 Following RNA binding step (step 7 in *DNA and RNA Purification*), add 420 µL **DNA/RNA Wash Buffer** to the green column, centrifuge (16,000 xG for 2 minutes) and discard the flow through.

13 For each RNA 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.

DNase I Reaction Mix:
DNase I: 5 µL
DNA Digestion Buffer: 75 µL

DNA and RNA Purification (cont.)

14 Add 400 µL **DNA/RNA Prep Buffer** to the columns and centrifuge (16,000 xG for 1 minute). Discard the flow-through.

14.1 Add 700 µL of **DNA/RNA Wash Buffer** to the columns (pipetting so the buffer runs down the sides of the tube).

14.2 Centrifuge at 16,000 xg for 1 minute. Discard flow-through.

14.3 Add 250 µL **DNA/RNA Wash Buffer** to the column.

14.4 Centrifuge at 16,000 xg for 1 minute. Discard flow-through.

14.5 Add 250 µL **DNA/RNA Wash Buffer** to the column.

14.6 Centrifuge at 16,000 xg for 3 minutes. Discard flow-through and transfer columns to new collection tubes.

14.7 Add 50-100 µL DNase/RNase-Free Water to the column and incubate at room temperature (on ice for RNA) for 5 minutes.
During incubation, prepare **Zymo-Spin III HRC Spin Filters**.
- Place Zymo-Spin III-HRC Filter in a Collection Tube and add 600 µL ZymoBIOMICS HRC Prep Solution.
- Centrifuge at 8,000 xG for 3 minutes.

14.8 After incubation, centrifuge columns containing DNase/RNase-Free water at 16,000 xg for 2 minutes. Discard column.

14.9 Transfer prepared **Zymo-Spin III-HRC Spin Filters** into labeled 1.5 mL LoBind tubes.

14.10 Transfer eluted DNA/RNA into Zymo-Spin IV-HRC Spin Filter.

14.11 Centrifuge at 16,000 xg for 3 minutes.

Post - Extraction

15 Nanodrop all samples

NOTE:

1) Keep all samples on ice.
2) Blank with DNase/RNase-Free Water.
3) Record in lab notebook and MVP master spreadsheet

16 (OPTIONAL for RNA) Add 2.5 µL of RiboLOCK to RNA samples.

17 Store DNA and RNA samples in -80°C freezer in appropriate box