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ZYMO RESEARCH

RNA - Seq Made Simple

Zymo-Seq RiboFree® Universal cDNA Kit

One for All. Universal rRNA Depletion for Any Organism.

Highlights

- **Universal Depletion:** Novel probe-free technology depletes rRNA from any organism.
- **Simplest Kit:** Prepare NGS-grade RiboFree® cDNA from total RNA in as little as 1.75 hours.
- **Automation Friendly:** Streamlined protocol for increased scalability.

Catalog Numbers:
R3001

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

Zymo-Seq RiboFree® Universal cDNA Kit	R3001 (12 prep)	Storage Temperature
cDNA Synthesis Reagent 1	24 µL	-80°C
cDNA Synthesis Reagent 2	120 µL	-80°C
Depletion Reagent 1	120 µL	-80°C
Depletion Reagent 2	120 µL	-80°C
Depletion Reagent 3	120 µL	-80°C
Depletion Reagent 4	24 µL	-20°C
Select-a-Size MagBead Concentrate	30 µL × 2	4 °C
Select-a-Size MagBead Buffer	1 mL × 2	4 °C
Zymo-Seq™ Wash Buffer	6 mL	Room Temp.
DNA Elution Buffer	1 mL	Room Temp.
DNase/RNase-Free Water	1 mL	Room Temp.
Instruction Manual	1	-

Specifications

- **RNA Input:** 10 – 1000 ng of total RNA.
  - For optimal results, please use the recommended 10-1000 ng input.
  - If an input below 10 ng is necessary, see **Appendix B** for additional considerations and recommended modifications.
- **Input Quality –** RNA should be free of DNA contamination and enzymatic inhibitors, with A260/A280 and A260/A230 **≥ 1.8**. RNA with lower purity ratios (A260/A280 and A260/A230) should be treated with DNase I and purified with the **RNA Clean & Concentrator™ (Cat. No. R1013)** prior to processing. RNA should be suspended in water, TE, or a low-salt buffer.
  - For optimal results, please use intact RNA (RNA Integrity Number or RIN ≥ 8.0) whenever possible.
  - For degraded RNA input, see **Appendix C** for additional considerations and recommended modifications.
- **Processing Time –** ~1.75 hours[1]
- **Equipment Needed (user provided) –** Thermal cycler with heated lid, magnetic stand for 0.2 mL PCR tubes, microcentrifuge for 0.2 mL PCR tubes and 1.5 mL microcentrifuge tubes, and a benchtop vortex mixer.

1 Estimated based on processing ≤ 8 samples at a time using ≥ 250 ng of total RNA input. Handling more samples simultaneously or using a lower input will require longer processing time.

Product Description

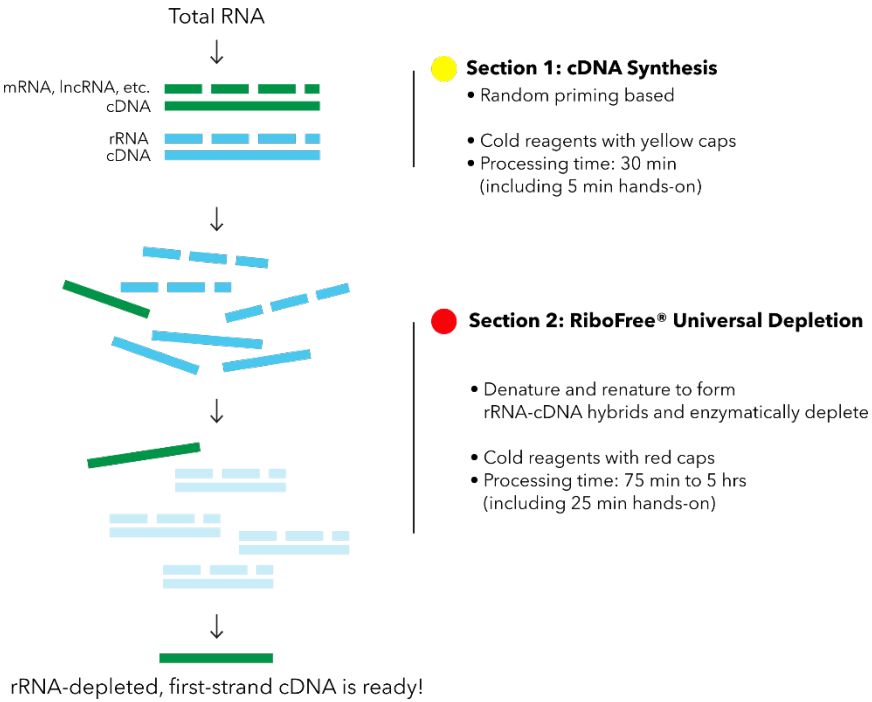
The **Zymo-Seq RiboFree® Universal cDNA Kit** is the simplest kit available for the preparation of ribosomal RNA (rRNA) depleted, NGS-grade cDNA.

Ribosomal RNAs (rRNA) can comprise approximately 90% of the total cellular RNA and represents an obstacle when analyzing unique protein coding sequences and other RNA of interest. This kit features RiboFree® Universal Depletion that uses the input RNA as templates to drive the depletion of the reverse transcribed cDNA from the highly abundant sequences. This eliminates the need for organism-specific probes that may require time-consuming customization and testing. This novel probe-free rRNA depletion strategy enables this kit to be compatible with total RNA from any species for subsequent qPCR detection, RNA-Seq library preparation, and any other downstream analysis**.**

This kit produces first-strand cDNA from any sample's full transcriptome (both coding and non-coding). In contrast to poly(A) targeted RNA pull-down or oligo (dT) priming, this kit produces cDNA from total RNA, including long-noncoding RNAs (lncRNA), intronic RNAs, nucleolar RNAs, and mRNAs with and without intact poly(A) tails.

The **Zymo-Seq RiboFree® Universal cDNA Kit** has a streamlined workflow minimizing user manipulation, resulting in an automation-friendly protocol with little hands-on time. See the next page for a detailed overview of RiboFree® Universal cDNA Preparation Procedure.

RiboFree Universal cDNA Kit Overview



Zymo-Seq RiboFree® Universal cDNA Kit Workflow. Processing time is estimated based on processing ≤ 8 samples at a time. Handling more samples simultaneously adds to the hands-on time and leads to a longer processing time.

Protocol

Buffer Preparation

- ✓ Prepare the **Select-a-Size MagBeads** at least 5 days before cDNA preparation.
    1. Add 30 µL of **Select-a-Size MagBead Concentrate** to every 1 mL of the **Select-a-Size MagBead Buffer.**
    2. Resuspend by pipetting up and down and vortexing. Store at 4°C to 8 °C.
- ✓ Add 24 mL of 100% ethanol (26 mL of 95% ethanol) to the 6 mL **Zymo-Seq™ Wash Buffer** concentrate provided.

Before Starting

- ✓ Set the thermal cycler lid temperature to 105°C for each program.
- ✓ Thaw and keep -80°C and -20°C components on ice during use. Mix reagents by flicking and centrifuge briefly.
- ✓ Allow the **Select-a-Size MagBeads** to equilibrate to room temperature for ≥30 minutes prior to use.
- ✓ Resuspend the magnetic particles immediately before each use by vigorously inverting and vortexing the **Select-a-Size MagBeads** until homogenous.
- ✓ Avoid multiple freeze-thaws of -80°C and -20°C components. Make aliquots as necessary.
- ✓ Use a multichannel pipette for reagent transfer to minimize hands-on time when processing multiple samples.
- ✓ **For new users, please read Appendix A: Select-a-Size MagBead Clean-up Protocol carefully before performing the protocol.**

Section 1: cDNA Synthesis (Yellow Caps)

1. Set up the following thermal cycler program (reaction volume: 20 µL).

Program	Step	Temperature	Time
Primer Annealing	1	98°C	3 min
	2	4°C	Hold
Reverse Transcription	3	25°C	5 min
	4	48°C	15 min
	5	4°C	Hold

2. Transfer the input RNA (10 – 1000 ng at a volume ≤ 8 µL)[1] into a 0.2 mL PCR tube. If the input volume is < 8 µL, use **DNase/RNase-Free Water** to raise the volume up to 8 µL. Place the sample on ice.

3. For input ≥ 50ng, add 2 µL of **cDNA Synthesis Reagent 1** to the sample tube for a total of 10 µL. For input < 50 ng, add 1 µL of **cDNA Synthesis Reagent 1** and supplement with 1 µL of **DNase/RNase-Free Water** to the sample tube for a total of 10 µL. Mix thoroughly by flicking or pipetting. Centrifuge briefly.

4. Place the tube in the thermal cycler and run **Steps 1-2** (**Primer Annealing**) of the program.

5. Add 10 µL of **cDNA Synthesis Reagent 2** to each sample during the 4°C hold (**Step 2**) or on ice. Mix thoroughly by pipetting. Centrifuge briefly.

6. Close the thermal cycler lid and continue **Steps 3-5** (**Reverse Transcription**) of the program.

7. Proceed directly to **Section 2**: **RiboFree® Universal Depletion** for the depletion of ribosomal RNA.

1 If using an input lower than the kit's recommended minimum (10 ng) or desiring to skip depletion, refer to **Appendix B** for additional considerations before continuing the protocol. If using degraded RNA as input, refer to **Appendix C** for recommendations before continuing the protocol.

Section 2: RiboFree® Universal Depletion (Red Caps)

1. Set up the following thermal cycler program (reaction volume: 50 µL). Adjust the "Depletion Reaction" time based on the RNA input amount as listed on the right side of the table[1]. *Please note that this section involves adding reagents promptly to tubes inside a thermal cycler.*

Program	Step	Temperature	Time
Pre-Depletion Incubation	1	98°C	3 min
	2	68°C	5 min
	3	68°C	Hold
Depletion Reaction	4	68°C	X min
Stop Depletion	5	68°C	Hold
	6	98°C	2 min
	7	25°C	Hold
Depletion Cleanup	8	55°C	15 min
	9	95°C	5 min
	10	25°C	Hold
DNA Elution	11	95°C	5 min
	12	25°C	Hold

≥ 250 ng: X = 15
150 ng: X = 30
100 ng: X = 60
50 ng: X = 90
25 ng: X = 120
10 ng: X = 240

2. Add 10 µL of **Depletion Reagent 1** to the tube containing the 20 µL sample from Section 1, Step 7 on ice for a total of 30 µL. Mix thoroughly by pipetting. Centrifuge briefly.

3. Place the tube to the thermal cycler and run **Steps 1-3** (**Pre-Depletion Incubation**) of the program. **DO NOT** remove the tube from the thermal cycler at the **Step 3** hold.

4. Without removing the tube, add 10 µL of **Depletion Reagent 2** to the 30 µL sample for a total of 40 µL. Mix thoroughly by pipetting.

Continue Section 2 protocol on the next page.

1 RiboFree® universal depletion of rRNA uses the input RNA as templates to drive the enzymatic digestion of reverse transcribed cDNA from the highly abundant rRNA sequences. Therefore, lower input requires longer depletion reaction time, and the listed duration serves as a starting point. For good practice, optimization of depletion reaction time is recommended, especially prior to working with precious samples.

5. Close the thermal cycler lid and continue **Step 4** (**Depletion Reaction**) of the program. **DO NOT** remove the tube from the thermal cycler at the **Step 5** hold.

6. Without removing the tube, add 10 µL of **Depletion Reagent 3** to the 40 µL sample for a total of 50 µL. Mix thoroughly by pipetting.

7. Close the thermal cycler lid and continue through **Steps 6-7** (**Stop Depletion**) of the program.

8. Add 2 µL of **Depletion Reagent 4** to the 50 µL sample for a total of 52 µL. Quickly remove the tube from the thermal cycler and gently flick to mix thoroughly. Centrifuge briefly to collect the reaction. Immediately return the tube to the thermal cycler to continue.

9. Close the thermal cycler lid and continue through **Steps 8-10** (**Depletion Cleanup**) of the program.

10. Remove the tube from the thermal cycler and centrifuge briefly. At room temperature, add 26 µL of 95% ethanol to the 52 µL sample for a total of 78 µL. Mix thoroughly by pipetting.

11. Follow the clean-up protocol (**Appendix A**, page 9) to obtain purified cDNA. **Steps 11-12** (**DNA Elution**) of the program is utilized for elution at the end of the clean-up protocol[1].

The eluate is your final RiboFree® first-strand cDNA, and may be stored at ≤ 4°C overnight or ≤ −20°C for long-term storage.

1 The side wall of the tube may have a brown shade from heating the beads. This is not a concern and the eluate will be clear after placing it on the magnet.

Appendix A: Select-a-Size MagBead Clean-up Protocol

Before Starting:

- ✓ Allow the **Select-a-Size MagBeads** to equilibrate to room temperature for ≥30 minutes prior to use.
- ✓ Resuspend the magnetic particles immediately before each use by vigorously shaking or vortexing the **Select-a-Size MagBeads** until homogenous.

1. Add 156 µL of **Select-a-Size MagBeads** to each sample. Mix thoroughly by pipetting until homogenous. Incubate for 5 minutes at room temperature.

2. Place the sample on a magnetic stand for 5 minutes, or until the beads have fully separated from the solution. Without dislodging the bead pellet, aspirate slowly and discard the supernatant.

3. While the sample is still on the magnetic stand, add 200 µL of **Zymo-Seq™ Wash Buffer** without disturbing the bead pellet. Aspirate slowly and discard the supernatant without dislodging the bead pellet. Repeat this step for a total of 2 washes.

4. While the sample is still on the magnetic stand, keep the tube cap open to air-dry the beads. After 1 minute, aspirate any residual **Zymo-Seq™ Wash Buffer** that has collected at the bottom of the tube. Continue to air-dry until the bead pellet appears matte without cracking. See an example image on the next page for reference.

5. Remove the sample from the magnetic stand. Add ≥10 µL of **DNA Elution Buffer** to the beads and mix thoroughly by pipetting up and down until homogenous.

6. Transfer the sample to the thermal cycler to complete **Steps 11-12** (**DNA Elution**) of the program in Section 2.

7. Remove the sample from the thermal cycler. Centrifuge briefly to collect all the eluate.

8. Place the sample on the magnetic stand for 1-2 minutes or until the eluate is clear. Transfer the eluate to a new tube for each sample.

Further Information on Bead Pellet Air-dry

The optimal air-dry time can vary depending on the humidity and temperature. Optimally dried beads should appear matte without cracking (e.g., the one in the middle in Figure 1 below).

Start with 5 minutes of air-dry time and adjust the time as needed to achieve optimally dried beads. Wash buffer carryover from insufficiently dried beads or overdried, cracked beads may reduce nucleic acid recovery.

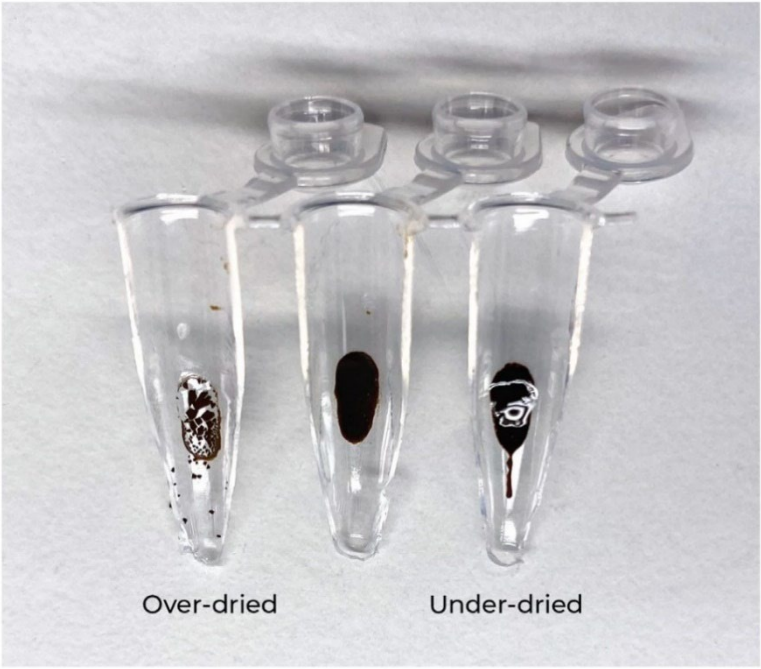


Figure 1. Over-dried beads are cracked and flakey, resembling dried mud. Under-dried beads are glossy and wet, like saturated mud. Optimally dried beads appear damp, but lack gloss.

Appendix B: Considerations for Low Input or No Depletion

For cDNA Preparation with Low Input (1 – 9 ng)

It is possible to prepare cDNA with input lower than the kit's minimum input requirement (10 ng); however, the rRNA depletion efficiency may be negatively impacted. Use the recommended modifications below if the total RNA input is below 10 ng.

1. For 5 – 9 ng of total RNA input:
   - At Section 1, Step 3 (page 6): Add 1 µL of **cDNA Synthesis Reagent 1** and supplement with 1 µL of **DNase/RNase-Free Water** to the sample tube for a total of 10 µL.
   - At Section 2, Step 1 (page 7): Adjust "Depletion Reaction" to be 240 mins (i.e., 4 hours).

2. For 1 – 4 ng of total RNA input:
   - At Section 1, Step 3 (page 6):
     a. Dilute 1 µL of **cDNA Synthesis Reagent 1** with 4 µL of **DNA Elution Buffer**.
     b. Add 1 µL of the diluted cDNA Synthesis Reagent and supplement with 1 µL of **DNase/RNase-Free Water** to the sample tube for a total of 10 µL.
   - At Section 2, Step 1 (page 7): Adjust "Depletion Reaction" to be 240 mins (i.e., 4 hours).

For cDNA Preparation without Depletion

It is possible to prepare cDNA without rRNA depletion using this kit. For such a need, please follow the recommended modifications below.

- Upon completing Section 1, Step 6 (page 6), add 32 µL of **DNase/RNase-Free Water** to the 20 µL reaction. Mix thoroughly by pipetting.
- Skip to Section 2, Step 10 (page 8) to continue the protocol with no further modifications.

Appendix C: Considerations for Degraded RNA Input

It is possible to prepare cDNA using degraded RNA with this kit; however, the rRNA depletion efficiency may be negatively impacted. Use the recommended modifications below if RNA input is degraded.

1. For RNA with a RIN ~4 or peak ≥ 500 nt:
    - Use more than the minimum input whenever possible.

2. For RNA with a RIN < 4 or peak < 500 nt:
    - Use ≥ 50 ng as input whenever possible.
    - At Section 1, Step 3 (page 6):
        a. Dilute 1 µL of **cDNA Synthesis Reagent 1** with 4 µL of **DNA Elution Buffer**.
        b. Add 1 µL of the diluted cDNA Synthesis Reagent 1 and supplement with 1 µL of **DNase/RNase-Free Water** to the sample tube for a total of 10 µL.

Ordering Information

Product Description	Catalog No.	Size
Zymo-Seq RiboFree® Universal cDNA Kit	R3001	12 preps
Zymo-Seq RiboFree® Total RNA Library Kit	R3000	12 preps
Zymo-Seq RiboFree® Total RNA Library Kit	R3003	96 preps

Individual Kit Components	Catalog No.	Amount
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10 W1001-30	1 ml 4 ml 6 ml 10 ml 30 ml
Zymo-Seq™ Wash Buffer	R3004-1-6 R3004-1-48	6 mL 48 mL
DNA Elution Buffer	D3004-4-10 D3004-4-50	10 mL 50 mL

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Featuring Universal rRNA Depletion for Any Organism

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Stranded Libraries

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Notes

Notes

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