E.Z.N.A.® Plant & Fungal RNA Kit

Revision No: 1.0

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

Product	R6827-00	R6827-01	R6827-02
Purifications	5	50	200
HiBind® RNA Mini Columns	5	50	200
Homogenizer Mini Columns	5	50	200
2 mL Collection Tubes	15	150	600
NTL Lysis Buffer	5 mL	40 mL	130 mL
SP Buffer	2 mL	10 mL	40 mL
RB Buffer	5 mL	30 mL	110 mL
RNA Wash Buffer I	5 mL	50 mL	200 mL
RNA Wash Buffer II	5 mL	25 mL	50 mL
Nuclease-free Water	2 mL	30 mL	60 mL

Supplied by user:

- Microcentrifuge capable of at least 14,000*g*
- Water bath, incubator, or heat block (for Difficult Samples protocol)
- RNase-free pipette tips and 1.5 mL microcentrifuge tubes
- 100% ethanol
- 70% ethanol
- 100% isopropanol (for Difficult Samples protocol)
- 2-mercaptoethanol (β-ME)
- Liquid nitrogen
- Disposable pestles

Before starting:

- For the Fresh/Frozen Protocol only, add 20 µL β-ME(14.3 M) per 1 mL RB Buffer.
- Prepare RNA Wash Buffer II according to the directions on the bottle.

RNA Extraction and Purification from Fresh or Frozen Plant/Fungal Tissue

LYSE

1. Collect tissue in a 1.5 mL microcentrifuge tube (not provided) and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable pestles. Do not allow samples to thaw.
2. Transfer up to 100 mg frozen ground plant or fungal tissue to a new 1.5 mL microcentrifuge tube. **Samples should not be allowed to thaw before the addition of RB Buffer in Step 3.**
3. Immediately add 500 µL RB Buffer mixed with 2-mercaptoethanol. Vortex at maximum speed to mix thoroughly. Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.
4. Insert a Homogenizer Mini Column into a 2 mL Collection Tube. Transfer the lysate to the Homogenizer Mini Column. Centrifuge at 14,000*g* for 5 minutes at room temperature. Discard the Homogenizer Mini Column.
5. Transfer cleared lysate to a new 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the pellet. Measure the volume of the lysate.

BIND

6. Add 1 volume 70% ethanol. Vortex at maximum speed for 20 seconds. A precipitate may form at this point; it will not interfere with DNA isolation. Passing the mixture through a needle using a syringe or by pipetting up and down may break up the precipitates.
7. Insert a HiBind® RNA Mini Column into a 2 mL Collection Tube.
8. Transfer 700 µL sample, including any precipitates that may have formed, to the HiBind® RNA Mini Column. Centrifuge at 12,000*g* for 1 minute at room temperature. Discard filtrate and reuse the collection tube.
9. Repeat Step 8 until all of the sample has been transferred to the column.

   OPTIONAL: This the starting point of an optional on-membrane DNase I Digestion protocol. If a DNA removal step is required, please continue to the DNase I Digestion Protocol on the reverse page. (See DNase I Digestion Set, Cat# E1091 for more information). If DNase I digestion is not required, proceed to Step 10.

WASH

10. Add 500 µL RNA Wash Buffer I. Centrifuge at 10,000*g* for 30 seconds. Discard the filtrate and the collection tube.
11. Transfer the HiBind® RNA Mini Column to a new 2 mL Collection Tube.

WASH

12. Add 700 µL RNA Wash Buffer II diluted with 100% ethanol (see bottle for instructions). Centrifuge at 10,000*g* for 30 seconds. Discard the filtrate and reuse the collection tube.
13. Repeat Step 12 with 500 µL RNA Wash Buffer II for a second wash step.
14. Centrifuge the empty HiBind® RNA Mini Column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.

ELUTE

15. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided). Add 50-100 µL Nuclease-free Water. Centrifuge at maximum speed for 1 minute. Store eluted RNA at -70°C.

RNA Extraction and Purification from Difficult Samples

LYSE

1. Collect tissue in a 1.5 mL microcentrifuge tube (not provided) and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable pestles. Do not allow samples to thaw.
2. Transfer up to 100 mg frozen ground plant or fungal tissue to a new 1.5 mL microcentrifuge tube. **Samples should not be allowed to thaw before the addition of NTL Lysis Buffer in Step 3.**
3. Immediately add 600 µL NTL Lysis Buffer mixed with 2-mercaptoethanol. Vortex at maximum speed to mix thoroughly. Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.
4. Add 140 µL SP Buffer. Vortex to mix thoroughly. Centrifuge at 10,000*g* for 10 minutes.
5. Transfer cleared lysate to a new 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet. Measure the volume of the lysate.
6. Add 1 volume 100% isopropanol. Vortex to mix thoroughly. Immediately centrifuge at 10,000*g* for 2 minutes.
7. Carefully aspirate or decant the supernatant. Do not disturb the RNA pellet. Invert the microcentrifuge tube on a paper towel for 1 minute to allow any residual liquid to drain. Drying the pellet is not necessary.
8. Add 100 µL RB Buffer or Nuclease-free Water heated to 65°C. Vortex at maximum speed to resuspend the pellet. A brief incubation at 65°C may be necessary to effectively dissolve the RNA. **Do not add 2-mercaptoethanol to RB Buffer for this step.** RB Buffer is recommended for dissolving the RNA pellet. Nuclease-free Water should only be used if dissolving the RNA with RB Buffer has proven difficult.

BIND

9. Adjust the binding conditions by following either **A or B** below. **RB Buffer must be mixed with 2-mercaptoethanol before use.**
   A. If RB Buffer was used in Step 8, add 250 µL RB Buffer and 350 µL 100% ethanol.
   B. If Nuclease-free Water was used in Step 8, add 350 µL RB Buffer and 250 µL 100% ethanol.
10. Proceed to Step 8 of the RNA EXTRACTION AND PURIFICATION FROM FRESH OR FROZEN PLANT/FUNGAL TISSUE protocol on the reverse page.

DNase I Digestion Protocol

1. For each HiBind® RNA Mini Column, prepare 75 µL DNase I stock solution (73.5 µL DNase I Digestion Buffer + 1.5 µL DNase I (20 Kunitz/µL)). See DNase I Digestion Set, Cat# E1091 for more information.
2. Add 250 µL RNA Wash Buffer I. Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and reuse the collection tube.
3. Add 75 µL DNase I digestion mixture directly onto the surface of the membrane of the HiBind® RNA Mini Column. DNA digestion will not be complete if some of the mixture is retained on the wall of the HiBind® RNA Mini Column.
4. Let sit at room temperature for 15 minutes.
5. Add 250 µL RNA Wash Buffer I. Let sit at room temperature for 2 minutes. Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and reuse the Collection Tube.
6. Proceed to Step 12 of the RNA EXTRACTION AND PURIFICATION FROM FRESH OR FROZEN PLANT/FUNGAL TISSUE protocol at the top of this page.