## Contents



**Revised September 2012**



### Introduction

E.Z.N.A.® Plant DNA/RNA Isolation Kits are designed for isolation of cellular RNA and genomic DNA simultaneously from single plant or fungal sample. Lysates are first passed through a E.Z.N.A.® DNA binding column to selectively isolate DNA and then through a E.Z.N.A.® RNA column to selectively isolate RNA. Unlike some of other procedures that either the sample or purified nucleic acid is divided sample into two parts before being processed separately. E.Z.N.A. Plant DNA/RNA Kit purify DNA and RNA from entire sample. There is no ultra-centrifugation, organic extractions, and ethanol precipitation steps involved in this protocol.

RNA purified using the E.Z.N.A.® DNA/RNA method is ready for applications such as RT-PCR*, qPCR*, differential display, microarrays, etc. DNA purified from this kit is suitable for PCR, Southern blot, genotyping and SNP analyses.

### Principle

The E.Z.N.A.® Plant DNA/RNA isolate kits combines reversible binding properties of HiBind® RNA technology with a a specially designed buffer system which selective bind DNA to a DNA column before RNA isolation. Samples are first lysed and homogenized in a specially designed denature buffer (RB-X), which immediately inhibit the activity of RNase and DNase. The lyste is then passed through a E.Z.N.A.® DNA Column which will selectively bind genomic DNA. After two quick wash steps, the purified DNA is eluted. The flow-through lysate from E.Z.N.A.® DNA Column is then added ethanol to create proper RNA binding condition, the sample is then loaded into the E.Z.N.A.® RNA Column to bind RNA. With a brief centrifugation or vacuum, the samples pass through the Column and the RNA binds to the Hibind™ matrix. After two wash steps, purified RNA is eluted with RNase-free water.

### Storage

All components in the E.Z.N.A.® DNA/RNA Kit should be stored at room temperature. During shipping and storage in cool ambient conditions, crystals may form in the Buffer PR. Simply warm the buffer to 37°C and gently shake its container to dissolve. All kit components are guaranteed for at least 12 months from date of purchase.

*The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 (and international equivalents) owned by Hoffmann-LaRoche, Inc.

## Kit Contents

| E.Z.N.A.® DNA/RNA Kits | R6733-00 | R6733-01 | R6733-02 |
|---|---|---|---|
| Purification times | 5 Preps | 50 Preps | 200 Preps |
| HiBind DNA Columns | 5 | 50 | 200 |
| HiBind RNA Columns | 5 | 50 | 200 |
| Collection Tubes | 15 | 150 | 600 |
| CPL Buffer | 5 ml | 50 ml | 200 ml |
| Buffer PR | 3 ml | 30 ml | 120 ml |
| RWC Wash Buffer | 5 ml | 50 ml | 200 ml |
| RNA Wash Buffer II | 2 ml | 12 ml | 4 X 12 ml |
| DNA Wash Buffer | 2 ml | 15 ml | 50 ml |
| DEPC-dd$H_2O$ | 2 ml | 15 ml | 30 ml |
| Elution Buffer | 1 ml | 15 ml | 30 ml |
| Instruction Manual | 1 | 1 | 1 |

### Important Notes

1. Please take a few minutes to read this booklet thoroughly to become familiar with the protocol. Prepare all materials required before starting the procedure to minimize RNA degradation.

- Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.

- Carefully apply the sample or solution to the HiBind® RNA membrane. Avoid touching the membrane with pipet tips.



## Before Starting

| IMPORTANT | |
|---|---|
| | **RNA Wash Buffer II Concentrate** must be diluted with absolute ethanol (96%-100%) before use: |
| | R6733-00 Add 8ml 96%-100% ethanol |
| | R6733-01 Add 48 ml 96%-100% ethanol |
| | R6733-02 Add 48 ml 96%-100% ethanol/bottle |
| | **DNA Wash Buffer** must be diluted with absolute ethanol (96%-100%) before use: |
| | R6733-00 Add 8 ml 96%-100% ethanol |
| | R6733-01 Add 60 ml 96%-100% ethanol |
| | R6733-02 Add 200 ml 96%-100% ethanol |

## E.Z.N.A.® Plant DNA/RNA Protocol

Materials to be provided by user:

- Centrifuge capable of 14,000 x g
- Water Bath or Heating Block set to 65°C
- RNase-free filter pipette tips
- 1.5 mL Centrifuge Tubes
- 2-mercaptoethanol
- Absolute ethanol
- Liquid nitrogen for freezing/disrupting samples
- Preheat elution buffer to 65°C

**NOTE:** Use extreme caution when handling liquid nitrogen.
This protocol is suitable for most fresh or frozen tissue samples allowing efficient recovery of RNA and DNA. However, due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to ≤100 mg plant tissue and ≤40 mg seed tissue. (Less starting material often results in better quality yields.) Best results are obtained with young leaves or needles. This method isolates sufficient RNA for a few tracks on a standard Northern assay, depending on the type and quality of the sample.

Wearing latex disposable gloves, collect tissue in a 1.5 ml or 2 ml microfuge tube

and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable pestles (available from OBI Cat# SS-1014-39 &1015-39) or equivalent. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70°C for later use. Do not allow samples to thaw. Use disposable pestles only once. Alternatively, a small clean mortar and pestle can be used. The above methods for disrupting plant tissue cannot be replaced with mechanical homogenizers.

**Note that all centrifugation steps must be carried out at room temperature.**

### Procedure:

1. **Add 500 µl Buffer CPL/2-mercaptoethanol per sample to a 1.5 mL Centrifuge tube.**

   **Note:** Add 20 µl 2-mercaptoethanol per 1 ml of Buffer CPL before use. This mixture can be made and stored at room temperature for 1 month. 2-mercaptoethanol should be added again (estimate same proportion) if Buffer CPL is stored for more than 1 month.

2. **Collect frozen ground plant tissue (up to 100 mg) or seed tissue (up to 30 mg) and transfer to a centrifuge tube containing Buffer CPL/2-mercaptoethanol.** Samples should not be allowed to thaw before adding to Buffer CPL/2-mercaptoethanol. We recommend starting with 30 to 50 mg plant tissue or 12 to 20 mg seed tissue. If results obtained are satisfactory, increase amount of starting material up to maximum limits. Vortex vigorously to make sure that all clumps are dispersed. RNA cannot be effectively extracted from clumped tissue.

   **Tip:** As a guide, a 2-cm diameter leaf square weighs approximately 100 mg.

3. Incubate at 55°C for 10 minutes.

4. Add **500 µl Chloroform to the sample. Vortex at maxi speed for 30 seconds.**

5. **Centrifuge the tube at 13,000 x g for 5 minutes.**

6. **Transfer 350 µl the cleared lysate into a new tube. Add 350 µl Buffer PR to the sample. Vortex to mix.**

7. **Transfer the mixture from step 6 to the HiBind DNA column inserted into a 2 mL collection tube(supplied).** Spin at 10,000 x g for 1 minutes at room temperature.



8. Store the E.Z.N.A.® DNA column at room temperature (15-25°C) or at 4°C for later DNA purification in steps 17-20. Use the flow-through for RNA purification in steps 9-16.

### Total RNA Purification

9. **Add 0.5 volume of absolute ethanol to the flow-through. Mix throughly by pipetting or vortexing.**(for miRNA Isolation, add 1.2 volume of absolute ethanol to the flow through. )

10. **Transfer 700 µl the sample to a E.Z.N.A. RNA column inserted into a 2 mL collection tube(supplied).** Spin at 10,000 x g for 1 minutes at room temperature. Discard the liquid.

11. Insert the column back into the collection tube. Transfer the remaining of the sample into RNA Column. Spin at 10,000 x g for 1 minutes at room temperature. Discard the liquid.

12. **Insert the column into a new collection tube and add 500µl RWC Wash Buffer directly onto the HiBind RNA Column. Centrifuge at 10,000 x g for 1 minutes at room temperature.** Discard the flow-though liquid and re-use the collection tube.

13. **Add 500 µl RNA Wash Buffer II directly onto the HiBind RNA Column. Centrifuge at 10,000 x g for 1 minutes at room temperature**.

    ***Note:*** *Wash Buffer II Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.*

14. **Discard the flow-through and add 500 µl of RNA Wash Buffer II**. Centrifuge at 10,000 x g for 2 minutes at room temperature. The prolonged centrifugation is necessary to dry the RNA column.

    *Note: It is very important to dry the E.Z.N.A.® RNA column completely before the elution step to remove residual ethanol that might otherwise interfere with downstream applications.*

15. **Elution of RNA: Remove the HIBind RNA column carefully without transferring any residual liquid. Insert the HiBind ® RNA column into a new 1.5 mL Centrifuge tube.(not supplied)**

16. **Add 40-70 µl DEPC-treated water to the column,** Incubate for 2 minutes at room temperature. Centrifuge at 14,,000 x g for 1 minutes at room temperature to elute RNA.

***Note:*** *Elution volume and number can vary according to user preference. To maintain a higher RNA concentration, first eluate can be used for second elution.*

### Genomic DNA Purification

17. **Place the E.Z.N.A.® DNA column into a 2 ml collection tube(supplied). Add 500µl DNA Wash Buffer to the column.** Centrifuge at 10,000 x g for 1 minutes at room temperature. Discard flow-through and re-use the collection tube for the next step.

18. Add another 500µl DNA Wash Buffer directly onto the HiBind DNA column Centrifuge at 14,000 x g for 2 minutes at room temperature. The prolonged centrifugation is necessary to dry the E.Z.N.A.® DNA column.

19. Elution of DNA: Place the E.Z.N.A.® DNA column onto a 1.5 mL Centrifuge tube(not supplied)

20. Add 50-100 µl of Elution Buffer preheated to 65°C to the column, Make sure to add Elution Buffer directly onto DNA matrix. Incubate for 2 minutes at room temperature. Centrifuge at 10000 x g for 2 minutes at room temperature to elute DNA.

## Troubleshooting Tips

| Problem | Cause | Suggestion |
|---|---|---|
| Low nucleic acid yield | Insufficient disruption and homogenization | • Make sure that the plant sample are completely lysed and homogenized |
| | RNA remains on the column | • Pre-heat DEPC-water to 65° C prior to elution.<br>• Incubate for 5 min with water prior to elution |
| | Column is overloaded | • Reduce quantity of starting material. |
| Clogged well on column | Insufficient disruption and homogenization | • Reduce amount of starting material |
| Degraded RNA | Source | • Do not freeze and thaw sample more than once.<br>• Follow protocol closely, and work quickly. |
| | RNase contamination | • Ensure not to introduce RNase during the procedure.<br>• Check buffers for RNase contamination. |
| Problem in downstream applications | Salt carry-over during elution | • Ensure RNA Wash Buffer II has been diluted with 4 volumes of 100% ethanol as indicated on bottle.<br>• RNA Wash Buffer II must be stored at room temperature.<br>• Repeat wash with Wash Buffer II. |
| | Inhibitors of PCR | • Use less starting material<br>• Repeat wash with Wash Buffer II or DNA wash Buffer. |
| DNA contamination | Too much starting material | • Reduce the cell number and repeat the extraction.<br>• Perform DNase digestion |
| Little or no DNA eluted | Lost DNA during process | • Make sure DNA wash Buffer is diluted with absolute ethanol with correct amount of ethanol<br>• use the pre-heated Elution Buffer (65°C) for DNA elution. |
| RNA contamination on genomic DNA elute | | • Add 50µl of RNase A (20mg/ml) to the DNA wash Buffer. |