Total RNA extraction from *Oryza sativa* leaf and *Solanum tuberosum* leaf using Ribospin™ Seed/Fruit

Experimental Conditions

Materials Required

- Ribospin™ Seed/Fruit (50 preps: 317-150)
- Tissuelyser II (85300, supplier Q) or another bead beating device
- Liquid nitrogen (LN_2)
- Absolute ethanol (C_2H_6O, CAS No.: 64-17-5, ≥99.0%)
- β-mercaptoethanol (C_2H_6OS CAS No.: 60-24-2, ≥99.0%)
- 1.5 ml or 2.0 ml microcentrifuge tube
- Vortex mixer
- Centrifuge (Max. speed 14,000 rpm or ≥10,000 x g)
- Pipette & sterile pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)
- Ice

Sample Information

Sample type:

Oryza sativa leaf

Solanum tuberosum leaf

- Sampling: after collecting fresh leaves, put them in a bag and seal it.
- How to store: store in -70 ˚C deep freezer
- Homogenizing: Tissuelyser II (85300, supplier Q)
- Conditions
  - Sample amount
    Oryza sativa leaf: 100 mg
    Solanum tuberosum leaf: 80 mg
  - Elution volume: 50 µl

Protocol

Before experiment

1. Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer RBW and RNW as indicated on the bottle.
2. Prepare DNase I reaction mixture just before step 6.
   - Prepare aliquot DNase I and thaw on ice.
   - Mix 2 µl DNase I with 70 µl Buffer DRB.

Sample preparation

1. After measuring 100 mg of *Oryza sativa* leaf sample and 80 mg of *Solanum tuberosum* leaf sample, put them into a 1.5 ml microcentrifuge tube. Then, it is rapidly frozen using LN_2.
2. Grind the frozen sample using Tissuelyser II.
   - *Oryza sativa* leaf samples: 30 Hz, 40 sec
   - *Solanum tuberosum* leaf samples: 30 Hz, 20 sec
3. The next step is according to protocol II (not protocol I) of Ribospin™ Seed/Fruit.

Ribospin™ Seed/Fruit Protocol (Protocol II)

*** For more details and methods, please refer to the handbook of Ribpsin™ Seed/ Fruit.**

1. Add 500 µl Buffer SL and 5 µl β-mercaptoethanol to the sample and vortex vigorously for 15 sec.
2. Incubate the mixture for 3 min at room temperature and centrifuge the lysate at 13,000 rpm for 1 min. Transfer 300 µl of the supernatant to a new 1.5 ml microcentrifuge tube (not provided).
3. Add 300 µl Buffer ML to the supernatant and vortex vigorously for 15 sec and transfer all of the mixture to EzPure™ Filter (yellow).
4. Centrifuge at 13,000 rpm for 1 min and transfer 500 µl of the pass-through to a new 1.5 ml microcentrifuge tube (not provided).
5. Add 250 µl absolute ethanol to the supernatant and mix it well by inversion. Apply all of the mixture into Column Type F (blue ring) and centrifuge at 13,000 rpm for 1 min.
6. Add 500 µl Buffer RBW to Column Type F and centrifuge at 13,000 rpm for 30 sec.
7. Apply 70 µl DNase I reaction mixture onto the center of Column Type F for gDNA digestion. Incubate for 10 min at room temperature.
8. Add 500 µl Buffer RBW to Column Type F and centrifuge at 13,000 rpm for 30 sec. Add 500 µl Buffer RNW to Column Type F and centrifuge at 13,000 rpm for 30 sec.
9. Centrifuge at maximum speed for an additional 1 min to remove residual wash buffer. Transfer Column Type F to a new 1.5 ml microcentrifuge tube (provided).
10. Add 50 µl Nuclease-free Water to the center of the membrane in Column Type F and centrifuge at 13,000 rpm for 1 min.

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Result

#	Sample	Conc. (ng/μl)	$A_{260/280}$	$A_{260/230}$	Yield (μg)
1	*Oryza sativa* leaf (100 mg)	183.4	2.13	2.35	**9.17**
2		173.4	2.14	2.38	**8.67**
3		164.3	2.15	2.35	**8.22**
4	*Solanum tuberosum* leaf (80 mg)	111.9	2.15	2.20	**5.56**
5		106.9	2.16	2.18	**5.35**

Table 1. The concentrations, yield and and purity of RNA extracted from 100 mg of *Oryza sativa* leaf and 80 mg of *Solanum tuberosum* leaf samples.

※ Absorbance measurement instrument: NanoDrop™ 2000/2000c (ND-2000, supplier T)

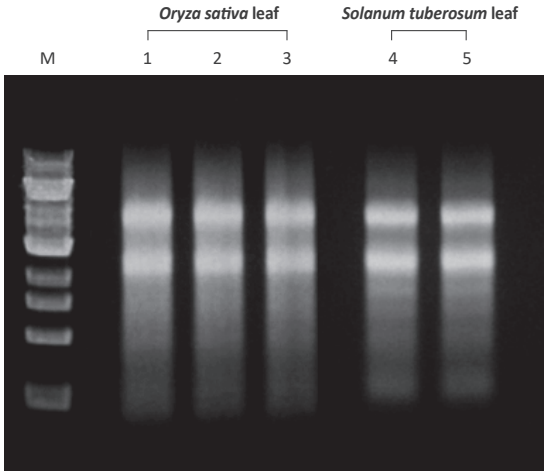

Figure 1. The result of electrophoresis of total RNA from 100 mg of *Oryza sativa* leaf and 80 mg of *Solanum tuberosum* leaf samples.
Lane M: GENESTA™ 1 kb DNA Ladder with 5X loading dye (GA-100, GeneAll®, 1 μl loading)
Lanes 1-3: 100 mg of *Oryza sativa* leaf samples (3 μl loading)
Lanes 4-5: 80 mg of *Solanum tuberosum* leaf samples (5 μl loading)

※ Electrophoresis conditions: 1.0% agarose gel (150 V, 17 min)