Ribospin™ Seed/Fruit is a specialized kit that enables high-quality total RNA extraction from various fruit samples

Experimental Conditions

Materials Required

- Ribospin™ Seed/Fruit (317-150, 50 preps)
- Grinder : blender, bead-beater or fruit grinding equipment with equivalent performance
- Liquid nitrogen (LN_2)
- Absolute ethanol (EtOH, C_2H_6O, CAS No. : 64-17-5, ≥99.0%)
- β-mercaptoethanol (β-ME, 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 (ex. lab coat, disposable gloves, goggles, etc.)
- Ice (prevents thermal damage of DNase I)

Sample Information

- Sample type : Fruit


Mango


Strawberry


Banana


Tomato


Apple

- Sampling :
  After collecting fresh fruit, put them in a bag and seal it.
- How to store : store at -70˚C temperature in a deep freezer
- Homogenizing : blender
- Extraction conditions
  - Sample amount : 100 mg
  - Elution volume : 50 µl

Protocol

Before Starting

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

Sample Preparation

1. Freeze the fruit samples using LN_2.
2. Grind the frozen samples using blender or another equipment.
3. Measure the weight of 100 mg and transfer the samples to 1.5 ml microcentrifuge tube.
4. The next step is according to **Ribospin™ Seed/Fruit protocol I** (not protocol II).

Ribospin™ Seed/Fruit Brief Protocol I

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

Lysis	1. Add 500 µl Buffer SL, Buffer ML, and 10 µl β-ME and vortex for 15 sec. 2. Incubate the mixture for 3 min at RT and centrifuge at 10,000 x g for 1 min.
Filtration	3. Transfer 600 µl of the supernatant to EzPure™ Filter. 4. Centrifuge at 10,000 x g for 1 min.
Binding	5. Transfer 500 µl of the pass-through to the new 1.5 ml microcentrifuge tube and add 250 µl of 100% EtOH. 6. Transfer all of the mixture to Column Type F. 7. Centrifuge at 10,000 x g for 1 min.
DNA digestion	8. Add 500 µl Buffer RBW to Column Type F. 9. Centrifuge at 10,000 x g for 30 sec. 10. Add 70 µl DNase I reaction mixture to Column Type F. 11. Incubate for 10 min at RT.
Washing	12. Add 500 µl Buffer RBW to Column Type F and centrifuge at 10,000 x g for 30 sec. 13. Add 500 µl Buffer RNW to Column Type F and centrifuge at 10,000 x g for 30 sec. 14. Centrifuge at full speed for 1 min and transfer Column Type F to the new 1.5 ml microcentrifuge tube.
Elution	15. Add 50 µl NFW to Column Type F and centrifuge at 10,000 x g for 1 min.

Table 1. Brief protocol of GeneAll® Ribospin™ Seed/Fruit kit for total RNA purification from fruit samples.

Ribospin™ Seed/Fruit is a specialized kit that enables high-quality total RNA extraction from various fruit samples

Result

#	Sample	Conc. (ng/μl)	Yield (μg)	$A_{260/280}$	$A_{260/230}$
1	Mango	89.70	**4.49**	2.17	2.16
2		93.90	**4.70**	2.17	1.82
3	Strawberry	23.20	**1.16**	1.94	1.10
4		21.80	**1.09**	1.94	0.86
5	Banana	24.90	**1.25**	2.12	0.94
6		29.40	**1.47**	2.09	1.13
7	Tomato	20.30	**1.02**	2.19	1.04
8		20.60	**1.03**	2.15	2.10
9	Apple	16.20	**0.81**	2.15	1.67
10		12.70	**0.64**	2.10	0.82

Table 2. The concentrations, yield and purity of total RNA extracted from 100 mg of various fruit samples.

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

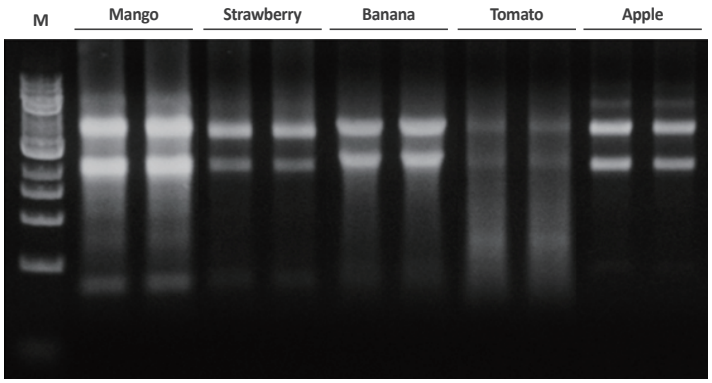

Figure 1. The result of electrophoresis of total RNA from 100 mg of various fruit samples.

Lane M : GENESTA™ 1 kb DNA Ladder with 5X loading dye (GA-100, GeneAll®, 1 μl loading)
※ Electrophoresis conditions : 1.0 % agarose gel, 150 V, 15 min, 7 μl loading