Total RNA extraction from *Ulva linza Linnaeus* samples using Ribospin™ Plant kit

Experimental Conditions

Materials Required

- Ribospin™ Plant kit (307-150, 50 preps)
- Tissuelyser II (85300, Q) (or another bead beating device)
- Liquid nitrogen (LN_2)
- Absolute ethanol (C_2H_6O, CAS No. : 64-17-5, ≥99.0%)
- 70% ethanol (C_2H_6O, CAS No. : 64-17-5, 70.0%)
- 1.5 ml or 2.0 ml microcentrifuge tube
- Vortex mixer
- Centrifuge (Max. speed 14,000 rpm)
- Pipette & sterile pipette tips
- Suitable protector (ex. lab coat, disposable gloves, goggles, etc.)
- Ice (for incubation)

Sample Information

- Sample type :

Ulva linza Linnaeus

- Sampling : After collecting fresh *Ulva linza Linnaeus* samples, 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 : 25, 50 and 100 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 8.
   - Prepare aliquot DNase I and thaw on ice.
   - Mix 2 µl DNase I with 70 µl Buffer DRB.

Sample preparation

1. After measuring 25, 50 and 100 mg of each sample, put it into a 1.5 ml microcentrifuge tube. Then, it is rapidly frozen using LN_2.
2. Grind the sample using TissueLyser II (30 Hz, 10 sec).
3. The next step is according to the protocol of Ribospin™ Plant.

Ribospin™ Plant brief protocol

*** For more details, refer to the handbook of Ribpsin™ Plant**

1. Transfer the powder into a 1.5 ml microcentrifuge tube.
2. Add 350 µl of Buffer RPL and incubate the mixture for 3 min at room temperature.
3. Transfer the lysate to EzPure™ Filter and centrifuge at ≥10,000 x g for 30 sec.
4. Transfer the supernatant into a 1.5 ml microcentrifuge tube.
5. Add 1 volume of 70% ethanol to the supernatant and mix well.
6. Apply the mixture into Column Type W and centrifuge at ≥ 10,000 x g for 30 sec.
7. Add 500 µl of Buffer RBW to Column Type W and centrifuge at ≥10,000 x g for 30 sec.
8. Apply the DNase I mixture into Column Type W.
9. Incubate the mixture for 10 min at room temperature.
10. Add 500 µl of Buffer RBW to Column Type W and incubate for 2 min and centrifuge at ≥10,000 x g for 30 sec.
11. Add 500 µl of Buffer RNW to Column Type W and centrifuge at ≥10,000 x g for 30 sec (twice).
12. Centrifuge at ≥10,000 x g for an additional 1 min.
13. Add 50 µl of Nuclease-free water to the center of the membrane.
14. Centrifuge at ≥10,000 x g for 1 min.

Result

#	Sample	Conc. (ng/µl)	$A_{260/280}$	$A_{260/230}$	Yield (µg)
1	*Ulva linza Linnaeus* (25 mg)	100.2	2.19	2.33	**5.0**
2		111.3	2.24	2.45	**5.6**
3	*Ulva linza Linnaeus* (50 mg)	187.7	2.21	2.24	**9.4**
4		183.6	2.25	2.42	**9.2**
5	*Ulva linza Linnaeus* (100 mg)	256.6	2.24	2.44	**12.8**
6		334.2	2.25	2.46	**16.7**

Table 1. The concentration, yield and and purity of RNA extracted from 25~100 mg of *Ulva linza Linnaeus* samples.

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

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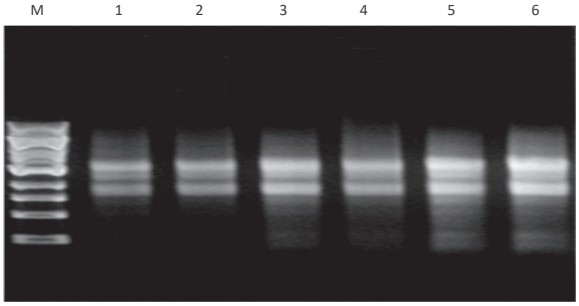

Figure 1. The result of electrophoresis of total RNA from *Ulva linza Linnaeus* sample.
Lanes 1~2 : 2 µl of eluate from 25 mg of *Ulva linza Linnaeus*
Lanes 3~4 : 2 µl of eluate from 50 mg of *Ulva linza Linnaeus*
Lanes 5~6 : 2 µl of eluate from 100 mg of *Ulva linza Linnaeus*
Lane M : GENESTA™ 1 kb DNA ladder with 5X loading dye (GA-100, GeneAll®, 1 µl loading)

※ Remark
Electrophoresis conditions : 1.0% agarose gel (150 V, 17 min, 2 µl loading)

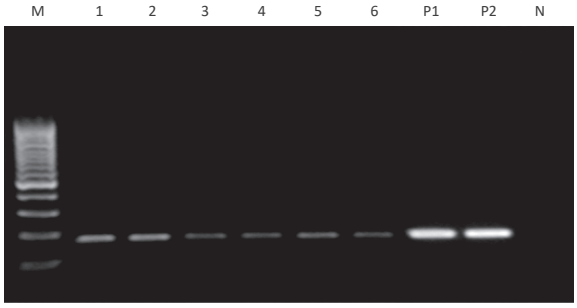

Figure 2. The result of electrophoresis of PCR products after RT-PCR.
Lanes 1~2 : 2 µl of eluate from 25 mg of *Ulva linza Linnaeus*
Lanes 3~4 : 2 µl of eluate from 50 mg of *Ulva linza Linnaeus*
Lanes 5~6 : 2 µl of eluate from 100 mg of *Ulva linza Linnaeus*
Lane M : GENESTA™ 100 bp DNA ladder with 5X loading dye (GA-010, GeneAll®, 1 µl loading)
Lanes P1~P2 : Positive control (total RNA template from pine leaf)
Lane N : Negative control (nuclease-free water)

※ Remark
Primer : NAd5 gene primer (gene of mitochondrial plant cell)
Electrophoresis condition : 2.0% agarose gel (150 V, 20 min, 3 µl loading)

Conclusion

The *Ulva Linnaeus* is well known for the wide distribution throughout the world and the research about its genomic information is widely conducted owe to its major contribution to biofouling.
The isolation of high-quality RNA from *Ulva Linnaeus* is the initial but the most import step for the successful research but there has always been problematic due to a high content a polyphenolic compounds and polysaccharides in the sample.
This study presents simple and effective method for the high-quality RNA isolation from *Ulva Linnaeus* in different starting amounts using Ribospin™ Plant Kit.
The RNA obtained from *Ulva Linnaeus* was found to be suitable for many downstream applications such as RT-PCR, PCR, qPCR, and etc.