Proposal of optimal sample amounts for high-quality total RNA extraction from sweet potato using the AllEx® Mini Plant Total RNA Kit and AllEx® Plant Total RNA Kit

Experimental Conditions

Materials

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AllEx® Mini Automated Nucleic Acid Extraction System [AEX012]	AllEx® Mini Plant Total RNA Kit [978-048]	AllEx®64 Automated Nucleic Acid Extraction System [AEX064]	AllEx® Plant Total RNA Kit [952-048, 952-096]

Sample & Extraction Information

Origin	*Ipomoea trifida* L.	
Sample		
	Sweet potato (*Ipomoea batatas* L.)	
Target	Total RNA	
Sample amount	100, 150, 200 mg	
Elution volume	Up to 100 µl	
Extraction system protocol	Plant RNA	
Operating time	AllEx® Mini	23’ 04’’
	AllEx®64	22’ 30’’

Protocol

AllEx® Mini Plant Total RNA Kit & AllEx® Plant Total RNA Kit Protocol

*** For more details and methods, please refer to the manual of AllEx® Mini Plant Total RNA Kit or AllEx® Plant Total RNA Kit.**

Preparation of DNase I Solution

To obtain a DNase I solution of 2 Kunitz units/µl, add 120 µl of Nuclease-free water to the tube containing lyophilized DNase I (240 Kunitz units), and mix carefully and gently to avoid foaming. The reconstituted enzyme should be stored at -20 ˚C for long-term stability.

Sample Preparation

1. Place 100, 150, or 200 mg of the samples into a 2 ml microcentrifuge tube containing a 5 mm stainless steel bead.
2. Grind each sample into a fine powder using a TissueLyser II (or an equivalent bead-beater) at 30 Hz for 1 min.
3. Add Buffer SQ1 to each sample in the follows:

Sample amount	Buffer SQ1
100 mg	400 µl
150 mg	500 µl
200 mg	600 µl

4. Vortex vigorously for 30 s and incubate for 5 min at room temperature.
5. Vortex vigorously and centrifuge at 13,000 rpm for 10 min at 4 ˚C.
6. Carefully transfer 300 µl of the supernatant to the 1st (7th) well of the cartridge, avoiding the starch and debris layer.
7. Add 4 µl of DNase I solution (2 Kunitz units/µl) to the 3rd (9th) well of the cartridge.

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Results

Sweet potato (*Ipomoea batatas* L.)	Mean (n=2)					
	AllEx® Mini Plant Total RNA Kit			AllEx® Plant Total RNA Kit		
	100 mg (CV)	150 mg (CV)	200 mg (CV)	100 mg (CV)	150 mg (CV)	200 mg (CV)
Yield (µg)	29.4 (0.14)	35.8 (0.03)	33.5 (0.01)	36.7 (0.25)	37.9 (0.06)	31.3 (0.13)
A_{260}/A_{280}	2.19 (0.00)	2.19 (0.00)	2.19 (0.00)	2.19 (0.00)	2.20 (0.00)	2.20 (0.00)
A_{260}/A_{230}	2.31 (0.01)	2.32 (0.00)	2.32 (0.01)	2.33 (0.01)	2.35 (0.01)	2.32 (0.01)

Table 1. Evaluation of RNA yield and purity from sweet potato
Total RNA was extracted from 100, 150, or 200 mg of sweet potato (n=2) using the AllEx® Mini Plant Total RNA Kit with the AllEx® Mini Automated Nucleic Acid Extraction System or the AllEx® Plant Total RNA Kit with the AllEx®64 Automated Nucleic Acid Extraction System, operating the Plant RNA protocol. The yield and purity of the extracted RNA were evaluated with a NanoDrop™ 2000 spectrophotometer.

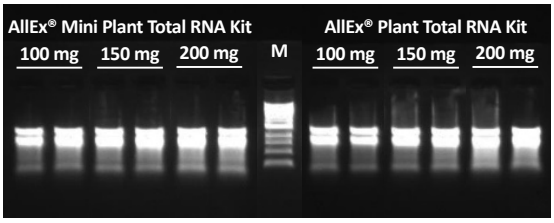

Figure 1. Agarose gel electrophoresis results of extracted total RNA from sweet potato
Extracted total RNA samples were subjected to electrophoresis on a 1% agarose gel in 0.5X TBE buffer at 150 V for 15 min. The extracted RNA size was calculated using the GENESTA™ 1 kb DNA Ladder (GA-100), and the electrophoresis results were analyzed with the SmartView Pro 1100 Imager System (UVCI-1100).

Conclusion

- Optimal pretreatment conditions and procedures were established for extracting high-quality total RNA from the starch-enriched tuberous root of sweet potato.
- If the sample is not sufficiently pulverized into powder during the grinding process, the extraction efficiency may be reduced. In such cases, please use liquid nitrogen to completely grind the sample into powder before proceeding to the lysis step.
- Ensuring the appropriate ratio (approximately 1:3) between the sample and Buffer SQ1 is essential for effective extraction.

Ordering Information

Cat. No.	Product	Size
AEX012	AllEx® Mini Automated Nucleic Acid Extraction System	1 Unit
978-048	AllEx® Mini Plant Total RNA Kit (Single Type)	48 T
AEX064	AllEx®64 Automated Nucleic Acid Extraction System	1 Unit
952-048	AllEx® Plant Total RNA Kit (Single Type)	48 T
952-096	AllEx® Plant Total RNA Kit (Plate Type)	96 T