iNtRON Biotechnology

AutoXT Plant RNA Kit (W/I)

For total RNA Extraction from various Plant tissues

Ver. INT-IFU-17605 (W/I)-R00

Cat. No

17605-96 (W); Well-plate type
17605-48 (I); Individual type

CONTENTS

INTRODUCTION

1. The AutoXT Plant RNA Kit is used in conjunction with the Miracle-AutoXT Nucleic Acid Extraction System (IMC-NC15PLUS) to purify total RNA from plant tissues such as leaves, stems, and seeds. This kit is designed for efficient and high-throughput RNA extraction, providing consistent and reliable results.

2. One of the key advantages of the AutoXT Plant RNA Kit is its streamlined workflow, enabling complete RNA extraction within 30 minutes, from sample preparation to final elution. Unlike some conventional RNA extraction methods, this protocol eliminates the need for phenol-based extraction and minimizes additional DNase treatment steps, making it both convenient and time-efficient.

3. The purified total RNA is of high quality, free from common plant-derived inhibitors such as polysaccharides and polyphenols, making it suitable for downstream applications such as PCR, qPCR, and next-generation sequencing (NGS). The Miracle-AutoXT Nucleic Acid Extraction System can process up to 32 samples simultaneously, maximizing efficiency in high-throughput settings.

PRODUCT COMPONENTS

Well plate type (W)

Contents	Unit	Q'ty
Prefilled Plates	16 test/Plate	6
Plunger Tips	ea	12
Lysis Buffer[A]	60 mL/bottle	1
DNA removal Columns[B]	48 ea/unit	2

Individual type (I)

Contents	Unit	Q'ty
Prefilled Cartridges	1 test/Cartridges	48
Plunger Tips	ea	12
Lysis Buffer[A]	30 mL/bottle	1
DNA removal Column[B]	48 ea/unit	1

A. This Buffer contains chaotropic salt.
B. Inserted into a collection tube (2.0 ml tube)

STORAGE CONDITIONS

AutoXT Plant RNA Kit should be stored dry, at room temperature (15–25°C). Under these conditions, AutoXT Plant RNA Kit can be stored for up to 24 months without showing any reduction in performance.

ADDITIONAL REQUIRED EQUIPMENT

AutoXT Plant RNA Kit provides all reagents for extracting RNA.
However, be prepared some equipment and reagents as follows for a fast and easy extraction.

- Miracle-AutoXT Automated Nucleic Acid Extraction System (IMC-NC15PLUS)
- Pipettes and pipette tips
- Vortex mixer
- Microcentrifuge with rotor for 2.0 ml tubes
- Microcentrifuge tubes (1.5 ml)
- Liquid nitrogen
- Other general lab equipment

SAFETY INFORMATION

The reagent Cartridges or Plates contain ethanol which is flammable. Guanidine thiocyanate and Guanidine hydrochloride (which are components of the Lysis Buffer and Washing Buffer 1) are harmful and irritants.
Always wear protective gear during handling chemical materials and the test should be handled by professionally trained person.

DO NOT add bleach or acidic solutions directly to the sample preparation waste.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days of purchase, and returning the product to iNtRON for examination.

CONSIDERATION BEFORE USE

1. Centrifugation : Centrifugation steps are carried out at 4 °C in a micro-centrifuge.

NOTICE

1. For research purpose only.

2. Always wear protective gear during handling chemical materials and the test should be handled by professionally trained person.

3. Be careful and prevent the contamination and direct contact from the test samples.

4. Surface of workspace and pipette should be regularly sterilized by 10% bleach solution.

5. All the waste should be sterilized before discarding.

SAMPLE PREPATION

Recommended Volume of Starting Materials according to Plant samples

Amounts of starting material for AutoXT Plant RNA Kit procedures

1. Determine the appropriate amount (50 mg-100 mg) of plant material. Do not use more than 100 mg.

Note : Weighing plant tissue is the most accurate way to determine the amount.

2. Transfer the plant sample to a mortar, add liquid nitrogen, and thoroughly grind it into a fine powder using a pestle.

Note : RNA in plant tissues is not protected until the tissues are flash-frozen in liquid nitrogen. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

3. Transfer the sample into a 1.5 mL micro tube and add 600 µL of Lysis Buffer. Vortex vigorously.

Note : Ensure the sample is finely ground or homogenized before adding Lysis buffer to maximize RNA extraction efficiency.

4. Pipette the lysate directly into a DNA removal Column placed in a 2 ml collection tube, and centrifuge for 1min at 13,000rpm.

Note : After centrifugation, check the collection tube to ensure all the lysate has passed through. If residue remains in the column, an additional short spin may be necessary.

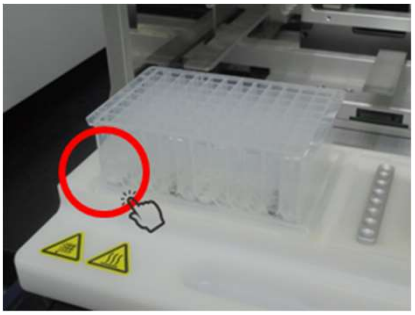
5. Transfer the 500 µL of flow-through into a new 1.5 mL microcentrifuge tube.

PROTOCOL

1. Add 500 µL of samples from [sample preparation Step] to each of the wells 1 and 7.

2. Insert Prefilled Well Plate or Cartridge Rack combined with Prefilled Cartridge on Heating Tray.

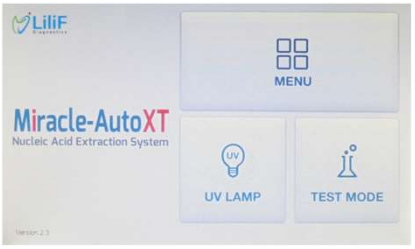
Note : Make sure the position of the diagonally cut edge of plat forward on the Heating Tray.

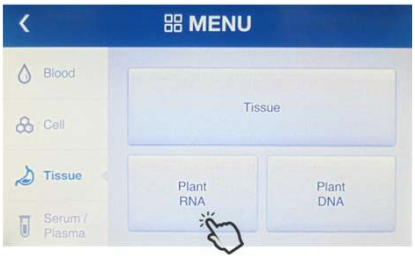


3. Close the front door and ready to start.

4. Press the 'menu / Tissue' button on the touch display of the Miracle-AutoXT Nucleic Acid Extraction System to select the extraction type.

5. Select 'Plant RNA' icon for Plant RNA extraction as shown figure below.





6. Press the 'Start' button to perform the extraction.

7. After completion of device working, transfer the 60~80 µL of Elution fraction (well position 6) to a new 1.5 ml Microtube.

TROUBLESHOOTING GUIDE

Problem	Possible Cause and Recommendation
Low RNA yield	- Inadequate tissue disruption. ensure proper grinding or homogenization before adding lysis buffer. Using liquid nitrogen, a bead beater, or a mechanical homogenizer can help finely disrupt tough plant tissues. Insufficient grinding may leave intact cells, reducing RNA release and affecting downstream applications. - Tissue has low RNA content. Some plant tissues have inherently low RNA content. For example, stems, woody tissues, and some mature leaves contain fewer nucleated cells compared to young leaves or seeds. Stems and vascular tissues are often composed of lignified cells or dead cells, which naturally have lower RNA yields. - Too much starting material. Using an excessive amount of starting material can overwhelm the lysis buffer, leading to incomplete cell disruption and reduced RNA yield. Overloaded samples may result in poor buffer penetration, leaving intact cells and causing inefficient RNA extraction. - Sample material not stored properly. Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid nitrogen. Samples should always be kept at -70°C. Never allow tissues to thaw before addition of Lysis buffer. Perform disruption of samples in liquid nitrogen. - The Miracle-AutoXT Nucleic Acid Extraction System Instrument was set for the wrong method. Ensure that the correct method is chosen in Plant RNA Mode. - Check that Plunger Tips were added to the plates/cartridges. Ensure that all plates/cartridges are snapped into the rack properly before processing.
Poor amplification	- Paramagnetic particle carryover may cause interference in amplification reaction. Remove particles in Elution Tube by centrifugation.
Cross-contamination	- Avoid splashing when adding lysates to plates/cartridges. Plates/Cartridges may be removed from the rack for sample addition to minimize contamination of adjacent plates/cartridges. Use fresh plastic wares for each sample to prevent sample-to sample contamination.
Plant method not an option on the instrument	- For the Miracle-AutoXT Nucleic Acid Extraction System Instrument, verify that the instrument is in Engineer mode. Verify that the instrument has firmware which includes the Plant RNA method.

EXPERIMENTAL INFORMATIONS

RNA extraction efficiency from different types of plant samples

The iNtRON's AutoXT Plant RNA Kit gave improved RNA yield compare with competitor from various plant samples.

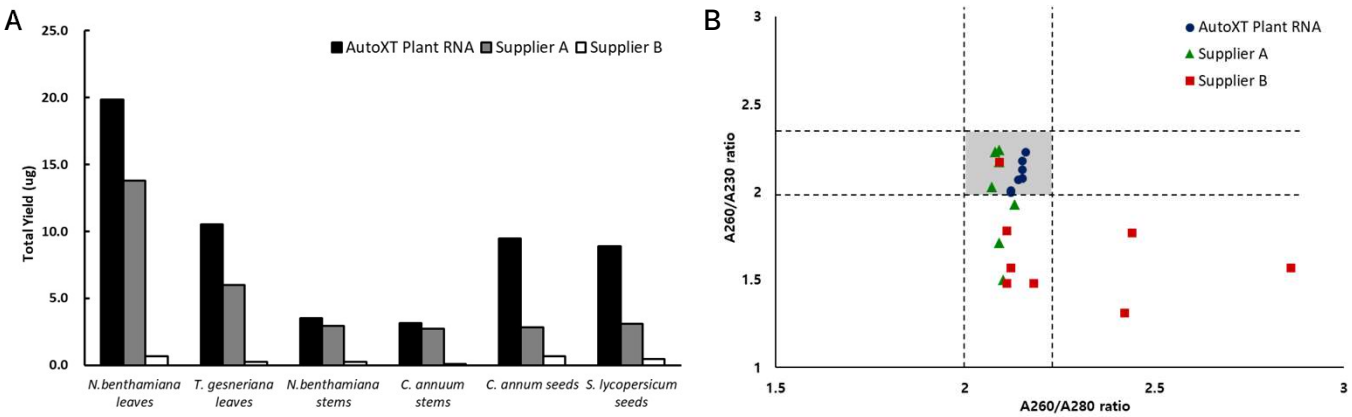


Fig. 1. Total RNA yield and purity were measured across various plant samples.
(A) Total RNA yield (µg) extracted from various plant samples using the AutoXT Plant RNA Kit (black bars), Supplier A (gray bars), and Supplier B (white bars). The AutoXT Plant DNA Kit consistently yields the highest DNA amounts across all tested plant tissues, including leaves, stems, and seeds.
(B) Scatter plot showing RNA purity, measured as A260/A280 vs. A260/A230 ratios, for samples extracted with competitor. The AutoXT Plant DNA Kit (blue dots) demonstrates high purity, clustering within the optimal range (A260/A280 ≈ 2.0-2.2, A260/A230 ≈ 2.0–2.3, shaded region), while competitor kits (Supplier A: green triangles, Supplier B: red squares) exhibit greater variability and lower purity values.

High quality of extracted RNA

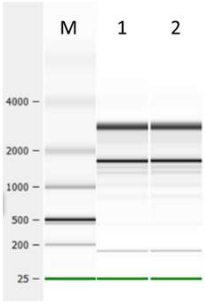


Fig. 2. Total RNA extracted using the AutoXT Plant RNA Kit was run on an Agilent 2100 Bioanalyzer and is displayed here as a gel electropherogram.
The electropherogram confirms the presence of intact total RNA bands with minimal degradation.
Lane M, RNA ladder marker; **Lane 1**, *L. sativa* seeds; **Lane 2**, *G. max* seeds.

Fig. 3. Electropherogram profiles of total RNA extracted from plant samples using AutoXT Plant RNA Kit.
The RNA Integrity Number (RIN) values were assessed to evaluate the integrity of the extracted RNA.
These results confirm that the AutoXT Plant RNA Kit effectively preserves RNA quality, making it suitable for downstream applications such as PCR, qPCR, and next-generation sequencing (NGS).
A, *L. sativa* seeds; **B**, *G. max* seeds.

EXPERIMENTAL INFORMATIONS

Determination of yield and purity data of various plant samples

The total RNA extraction results using AutoXT Plant RNA Kit were shown high quality and quantity of RNA collected from 50 mg of various plant tissue samples.

Type	Sample	Lane	RNA yield (μg)	A260/280 ratio
Leaves	*N. benthamiana*	1	7.14	2.08
	N. tabacum	2	6.82	2.11
	C. unshiu	3	4.68	2.12
	C. melo	4	6.92	2.11
	C. pepo	5	11.09	2.14
	C. quinoa	6	10.54	2.15
	C. annuum	7	7.83	2.11
	C. amaranticolor	8	19.86	2.15
	D. stramonium	9	10.58	2.11
	S. lycopersicum	10	11.83	2.15
	O. Sativa	11	8.07	2.11
	T. gesneriana	12	22.65	2.16
Stems	*N. benthamiana*	13	2.11	2.09
	N. tabacum	14	3.39	1.99
	C. annuum	15	3.52	2.05
	C. melo	16	3.13	2.08
	C. amaranticolor	17	4.96	2.04
	S. lycopersicum	18	2.95	1.96
Seeds	*N. benthamiana*	19	6.67	1.91
	C. annuum	20	8.04	2.09
	C. sativus	21	9.49	2.07
	H. vulgare	22	3.51	1.99
	S. lycopersicum	23	8.87	2.13
	G. max	24	60.99	2.16

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