GenUP™ Plant RNA Kit

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

PRODUCT	GenUP™ Plant RNA Kit		
CAT. NO.	BR0701501	BR0701502	BR0701503
SIZE	10 preps	50 preps	250 preps
COMPONENTS			
Buffer LYSIS LR	15 ml	30 ml	160 ml
Buffer LYSIS LT	8 ml	30 ml	130 ml
Buffer WASH A (concentrate)	5 ml (add 5 ml ethanol)	15 ml (add 15 ml ethanol)	70 ml (add 70 ml ethanol)
Buffer WASH B (concentrate)	6 ml (add 24 ml ethanol)	16 ml (add 64 ml ethanol)	2 × 36 ml (add 144 ml ethanol)
Water, RNase-free (for ELUTION)	2 ml	3 × 2 ml	25 ml
Mini Filters DNA (blue)	10	50	5 × 50
Mini Filters RNA (violet)	10	50	5 × 50
Collection Tubes (2.0 ml)	60	6 × 50	30 × 50
Elution Tubes (1.5 ml)	10	50	5 × 50

STORAGE

Room temperature (until expiry date – see product label).
If precipitation appears, gently warm the solution to dissolve the precipitate.

FEATURES

- Fast and simple procedure
- High-quality RNA isolated from a wide variety of plant samples
- Physical removal of DNA, no DNase treatment, no toxic β-mercaptoethanol

APPLICATIONS

- Isolation of total RNA from up to 100 mg plant material

DESCRIPTION

biotechrabbit™ GenUP Plant RNA Kit has been developed for quick and easy purification of total RNA from plant materials. After initial homogenization and lysis, genomic DNA is bound to a Mini Filter DNA, which can be discarded. RNA is selectively bound to a Mini Filter RNA, washed with two different buffers and eluted. The purified RNA is ready for use in any demanding molecular biology application, including RT-PCR.
Two lysis buffers, Buffer LYSIS LR and Buffer LYSIS LT, are provided to maximize yield. Most plant material can be processed with Buffer LYSIS LR. In the cases that yield using Buffer LYSIS LR is low, use Buffer LYSIS LT.

SPECIFICATIONS

STARTING MATERIAL	Plant material (up to 100 mg)
EXTRACTION TIME	30 min after homogenization
BINDING CAPACITY	Approximately 100 µg RNA
TYPICAL YIELD	Variable depending on the starting material; approximately 70 µg RNA
AVERAGE PURITY	A_{260}/A_{280} 1.7–2.0

MATERIALS SUPPLIED BY THE USER

- 96–99.8% ethanol
- 70% ethanol
- Reaction tubes
- Pipet tips
- Optional: DNase I

STEPS BEFORE STARTING

- Add the following volume of 96–99.8% ethanol to the indicated buffer bottle, close firmly, mix thoroughly and store at room temperature.

CAT. NO.		CONCENTRATE	ETHANOL	FINAL VOLUME
Buffer WASH A	BR0701501	5 ml	5 ml	10 ml
	BR0701502	15 ml	15 ml	30 ml
	BR0701503	70 ml	70 ml	140 ml
Buffer WASH B	BR0701501	6 ml	24 ml	30 ml
	BR0701502	16 ml	64 ml	80 ml
	BR0701503	36 ml	144 ml	180 ml

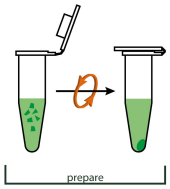
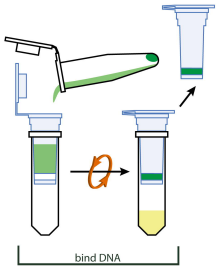
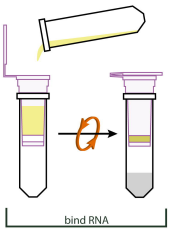
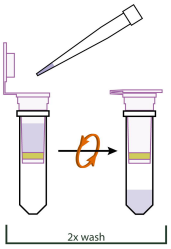
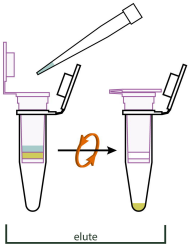
- Avoid repeated freezing and thawing of starting materials.
- Mark all vials and filters to avoid confusion when purifying multiple preps.
- Centrifugation steps should be carried out at room temperature.

GUIDELINES FOR PREVENTION OF RNA DEGRADATION

Special care should be taken to minimize contamination with RNases, as RNA is extremely sensitive to degradation.

- Always wear gloves, and change them frequently.
- Keep all tubes closed when possible.
- Keep samples and isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free).
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All glassware should be treated before use to ensure that it is RNase-free.
  - Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240 °C for four or more hours before use. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware.
  - Autoclaving alone will not inactivate many RNases completely. The glassware should be immersed in 0.1% diethylpyrocarbonate (DEPC) solution for 12 h at 37°C before autoclaving or heating to 100°C for 15 min to remove residual DEPC.
- Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-free water, rinsed with ethanol and finally allowed to dry.
- All buffers must be prepared with DEPC-treated RNase-free double-distilled water.
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

SHORT PROTOCOL

STEPS	SCHEME
• Homogenize plant material and lyse with Buffer LYSIS LR or Buffer LYSIS LT. • Centrifuge to remove unlysed material.	
• Transfer the lysate to the Mini Filter DNA (blue). • Centrifuge, and discard the Mini Filter DNA. • The filtrate contains the RNA, do not discard.	
• Add ethanol to the filtrate. • Transfer to a Mini Filter RNA (violet) to bind RNA and centrifuge.	
• Add Buffer WASH A and centrifuge. • Add Buffer WASH B and centrifuge. • Centrifuge once more to remove residual ethanol.	
• Elute RNA with Water, RNase-free (for ELUTION), and centrifuge. • Purified RNA in the Elution Tube is ready for use.	

PROTOCOL FOR ISOLATION OF TOTAL PLANT RNA

PROCEDURE	NOTES
• Homogenize up to 100 mg plant material completely. • Transfer 450 µl Buffer LYSIS LR or Buffer LYSIS LT to a 1.5 ml reaction tube. • Transfer the homogenized material to the tube containing the lysis buffer.	• For homogenization, use liquid nitrogen with a pestle and mortar or a rotor-stator homogenizer. When using liquid nitrogen, do not let the sample thaw. When using a rotor-stator homogenizer, the plant material can be homogenized in the lysis buffer. • The lysate can be stored at –20°C.
• Centrifuge at maximum speed for 1 min to pellet debris.	
• Transfer the supernatant to a Mini Filter DNA (blue) placed in a Collection Tube. • Discard the reaction tube. • Centrifuge at 10,000 × g (12,000 rpm) for 2 min. • Discard the Mini Filter DNA.	• If the solution does not pass completely through the Mini Filter, repeat the centrifugation or use a longer centrifugation time. • Do not discard the Collection Tube containing the RNA.
• Add an equal volume of 70% ethanol (400 µl) to the filtrate and mix by pipetting.	
• Transfer sample to the Mini Filter RNA (violet) placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min. • Discard the Collection Tube with the filtrate.	• If the solution does not pass completely through the Mini Filter, repeat the centrifugation or use a longer centrifugation time.
• Place the Mini Filter RNA into a new Collection Tube. • Add 500 µl Buffer WASH A to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH A as described above.
• Place the Mini Filter RNA into a new Collection Tube. • Add 650 µl Buffer WASH B to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH B as described above.
• Place the Mini Filter RNA into a new Collection Tube. • Add 650 µl Buffer WASH B to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	
• Place the Mini Filter RNA into a new Collection Tube. • Centrifuge at maximum speed for 2 min to remove residual ethanol. • Discard the Collection Tube.	
• Place the Mini Filter RNA into a new Elution Tube. • Add 30–80 µl Water, RNase-free (for ELUTION). • Incubate at room temperature for 1 min. • Centrifuge at 6000 × *g* (8000 rpm) for 1 min. • Discard the Mini Filter RNA.	• To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION). The minimum elution volume should exceed 20 µl.
• Purified RNA in the Elution Tube can be used immediately.	• Store the RNA at 4°C (short-term) or –20°C (long-term).

TROUBLESHOOTING

PROBLEM	SOLUTION
CLOGGED MINI FILTER	
Insufficient disruption or homogenization	Reduce the amount of starting material. After lysis, centrifuge lysate to pellet debris and continue the protocol using the supernatant.
LOW YIELD	
Insufficient disruption or homogenization	Reduce the amount of starting material. Do not overload the Mini Filter DNA.
Incomplete elution	Increase the elution time up to 5 min, or repeat the elution. Use a higher elution volume or elute in two steps.
DNA CONTAMINATION	
Too much starting material	Reduce the amount of starting material.
Incorrect lysis of starting material	Use the recommended technique to lysis the starting material. Perform an on-filter DNase digestion after binding RNA to the Mini Filter RNA. Alternatively, perform DNase digestion on the eluate.
TOTAL RNA DEGRADED	
Starting material handled or stored in appropriately	Ensure the starting material is fresh. Perform the homogenization and lysis steps quickly.
RNase contamination	Use sterile, RNase-free filter tips. Ensure pipets, devices and workplace are clean. Wear gloves.
PURIFIED RNA DOES NOT PERFORM WELL IN DOWNSTREAM APPLICATIONS (E.G., RT-PCR)	
Ethanol carried over to elution	Increase the centrifugation time when removing ethanol.
Salt carried over to elution	Ensure that Buffer WASH A and Buffer WASH B are at room temperature. Dissolve precipitates in the washing solutions by warming carefully.

SAFETY PRECAUTIONS

- This kit is made for single use only!
- Don't eat or drink components of the kit!
- The kit shall only be handled by educated personal in a laboratory environment!
- Wear gloves while handling these reagents, and avoid skin contact! In case of contact, flush with water immediately!
- Handle and discard waste according to local safety regulations!
- Do not add bleach or acidic components to the waste after sample preparation!
- Buffers LYSIS LR and WASH A contain guanidine isothiocyanate, which is harmful to health. Contact with acids liberates very toxic gas!

CERTIFICATE OF ANALYSIS

The components of the kit were tested for isolation of total RNA from plant material and subsequent analysis.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)/Sicherheitshinweise finden Sie in den SDS unter: http://www.biotechrabbit.com/support/documentation.html.

USEFUL HINTS

- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

CONTACT BIOTECHRABBIT

biotechrabbit GmbH
Volmerstr. 9a
12489 Berlin, Germany

info@biotechrabbit.com
support@biotechrabbit.com
www.biotechrabbit.com

Phone: +49 30 555 7821-10
Fax: +49 30 555 7821-99

Certified Quality-System mdc ISO 13485 ISO 9001

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valid from 24.08.2016