ambion® | RNA
by life technologies™

Ambion® Plant RNA Isolation Aid

Rapid Isolation of Total RNA from Plants

IMPORTANT! Before using this product, read and understand the "Safety Information" appendix in this document.

Introduction

Ambion® Plant RNA Isolation Aid is designed for removal of common contaminants of plant RNA preparations, such as polysaccharides and polyphenolics. It is compatible with most RNA isolation procedures that use chaotropic salts for lysis.

Use of the Plant RNA Isolation Aid adds just one additional centrifugation step in addition to the normal RNA isolation protocol. Tissue is homogenized in a mixture of the Plant RNA Isolation Aid and the lysis solution. Next, the preparation is clarified by centrifugation to remove the Plant RNA Isolation Aid and any insoluble material. RNA is then purified from the lysate according to the normal protocol.

The Plant RNA Isolation Aid has been validated using Ambion RNAqueous® and *mir*Vana™ miRNA Isolation Kits.

life technologies™

Contents, Storage and Stability

Contents

10 mL Plant RNA Isolation Aid

Storage and Stability

Store Plant RNA Isolation Aid at room temp or at 4°C.

Required Materials Not Provided

Reagents for tissue disruption

We recommend the Ambion RNAqueous Kit (P/N AM1912) for total RNA isolation, or the mirVana miRNA Isolation Kit (P/N AM1560) for quantitative recovery of small RNAs.

Tissue disruption equipment:

There are several options for tissue disruption; select a method based upon the particular needs of your experiment.

- Liquid nitrogen, mortar and pestle can be used to freeze and pulverize tough or fibrous tissue. Porcelain mortar and pestles are recommended.
- Motorized rotor/stator-type homogenizers: these versatile instruments effectively disrupt most types of tissue.
- Manual homogenizers: conical ground glass tissue grinders are recommended.
- Other options: electric blenders, Eberbach grinders, coffee grinders, and bead mills may also be effective, depending on the tissue.

Plant RNA Isolation Aid Procedure

1. Determine the appropriate tissue disruption method, and assemble the required equipment.
   - Relatively soft, non-fibrous tissue such as new leaves, blossoms, succulents, and seedlings can usually be homogenized fresh. In many cases, small pieces of fibrous tissue may also be used "fresh" rather than frozen, but more time and effort may be required to achieve adequate disruption.
   - Hard, fibrous tissues, such as seeds, woody stems, needles, and bark, often require snap-freezing in liquid nitrogen followed by pulverization in liquid nitrogen using a chilled porcelain mortar and pestle, blender, or coffee grinder.
2. Weigh the tissue sample.

   The upper limit of plant tissue that can be used varies significantly among plant types and between different isolation methods.
3. Homogenize tissue using 1 volume of Plant RNA Isolation Aid per unit mass of fresh tissue (mL/gm) and the amount of lysis solution called for by your isolation procedure.

   For example, for a 0.2 g tissue sample, process in 0.2 mL Plant RNA Isolation Aid plus the volume of lysis solution specified by your RNA isolation procedure.
   - Use at least 200 µL of solution for homogenization of small samples (25 µL Plant RNA Isolation Aid, and 175 µL lysis solution).
   - Fresh tissue can be minced with a scalpel or scissors prior to thorough homogenization if desired.
   - The optimal time and force needed to reduce plant tissue to a single-cell homogenate will vary with the tissue sample and the homogenizer used. Process until very little or no particulate matter is visible. For some tissues, the homogenate will appear relatively clear and free of particles, while for others it will not be possible to achieve this level of homogenization.

- When using a motorized homogenizer, the vessel capacity should be 2–3 times the sample volume to allow for foaming. Use short pulses of the homogenizer to minimize foaming.
- Frozen tissue that has been powdered should be mixed with lysis solution and then thoroughly homogenized.

4. Centrifuge the lysate to clarify.
   a. Centrifuge the lysate at top speed (10,000–15,000 x g) in a microcentrifuge for 5 min at room temp. This centrifugation will pellet insoluble debris and the Plant RNA Isolation Aid with its associated compounds.
   b. Transfer the supernatant to a new vessel; discard the pellet.
5. Continue RNA purification from the supernatant.

   The supernatant contains the RNA. Continue with your standard RNA purification procedure. If using the Ambion RNAqueous Kit proceed from section III.C; if using the mirVana miRNA Isolation Kit, proceed from section II.E.1.

Troubleshooting Plant RNA Isolation

This section provides suggestions for dealing with problems often encountered with plant RNA isolation. For further troubleshooting of RNA isolation, see the Ambion RNAqueous Kit Protocol.

Low RNA yield

1. Perform a mixing experiment

   The purpose of a mixing experiment is to determine whether low RNA yield is due to inadequate tissue disruption, or to the effects of contaminants, such as polysaccharides or phenolics. The strategy in this experiment is to compare yields from an "easy" reference tissue, to yields when the reference tissue is mixed with the problem tissue. Fresh alfalfa sprouts serve well as a reference tissue and are available year-round from grocery stores.
   a. Perform two RNA isolations; one using 150 mg of alfalfa sprouts, and the second, using 150 mg of alfalfa sprouts, mixed with 50 mg of the problem tissue.
   b. Elute both RNA samples in an equal volume.
   c. Evaluate RNA yield.

If the RNA yield in the mixed-tissue sample is significantly lower than that in the reference sample, the poor yield may be due to contaminants (e.g., polysaccharides, phenolic compounds, or other secondary metabolites) in the problem tissue.

If RNA yield from the reference sample is not compromised in the mixed-tissue sample, poor yield from the problem tissue is likely due to inadequate tissue disruption or poor sample quality (e.g., RNase degradation in the sample prior to RNA isolation).

2. Inadequate tissue disruption
   - To monitor cell disruption, the homogenate can be examined under a light microscope to directly see whether cells are intact or ruptured (Wilkins and Smart).
   - If fresh tissue was used, try snap-freezing and powdering the tissue in liquid nitrogen before disrupting.
   - To distinguish between low yield due to inadequate disruption versus problems due to release of RNases or other contaminants, perform the mixing experiment described above.

3. Tissue has low RNA content

   Some plant tissues have a low RNA content. For example, *Arabidopsis* has been reported to yield consistently low amounts of RNA from all tissues, and root tissue is reported to have lower-than-average RNA content (Wilkins and Smart). Generally, mature leaf tissue has a lower RNA content than young leaves.

4. Tissue has high levels of RNases, phenolics, or other contaminants

   To obtain good yields of high quality RNA from problematic tissues such as pine needles or mature cotton tissues (Baker et al. 1990), a more rigorous RNA isolation method may be required; for example, ultracentrifugation through cesium chloride. To distinguish between low yield due to contaminants, versus low yield due to inadequate tissue disruption, perform the mixing experiment described above.

5. The preparation is too dilute

   Yields may be improved in some cases by keeping the preparation more concentrated, especially if the tissue has a high water content. Try reducing the amount of lysis solution.

Significant errors in weighing small amounts of tissue (<100 mg) can be introduced if the tissue is extremely wet. Tissue samples should be blotted dry just before weighing.

Watery tissues may yield more RNA if they are lyophilized before disruption. This increases the ratio of lysis solution to dry-weight tissue (Wilkins and Smart 1996).

Contamination with polysaccharides

An A_{260}/A_{230} ratio of <2, indicates possible polysaccharides carryover (Cheng and Seeman 1998). There are many ways to decrease polysaccharide contamination of RNA, these include the following:

- Lithium chloride precipitation of RNA: For more information, go to **www.invitrogen.com/site/us/en/home/support/technical-support.html**. Lithium chloride solution and instructions for its use are included in the RNAqueous Kit.
- Potassium acetate precipitation of polysaccharides: adjust the RNA solution to 0.2 M potassium acetate, mix well, incubate on ice for 15 min, and then pellet the insoluble material for 10 min at 12,000 x g at 4°C (Wilkins and Smart 1996). RNA remains in the supernatant.
- Precipitation of polysaccharides with 30% ethanol at low salt concentration: used for grape berry tissue (Tesniere and Vaydat 1991).
- Precipitation of polysaccharides with 20% ethanol and 0.5 M potassium acetate: used for mango mesocarp (Lopez-Gomez and Gomez 1992).

Contamination with PCR inhibitors

Phenolic compounds are known to inhibit PCR, and they are especially difficult to remove from RNA because they can cross-link RNA under oxidizing conditions (Wilkins and Smart 1996). If no RT-PCR product can be amplified from an RNA preparation, perform a mixing experiment to check for inhibitors of the RT-PCR: Reverse transcribe RNA from a positive control that is known to function well in PCR, and the test sample. Set up three PCRs using cDNA reverse transcribed from the two different RNAs as follows:

1. a positive control
2. the questionable RNA
3. an equal mix of 1 and 2

If there is no product from reaction 3, but reaction 1 gives the expected product, this indicates the presence of an inhibitor in the test sample.

The effects of an inhibitor can be mitigated by using less RNA in the reverse transcription reaction. The lower limit of sample needed for the reaction is dictated by the abundance of the target and the efficiency of the PCR.

It may be possible to reduce the inhibitory effect of polyphenolic contamination, by inclusion of PVP in the PCR (Koonjul et al. 1999). Plant RNA Isolation Aid, which contains PVP, can be used for this purpose; add 1.25–5 µL Plant RNA Isolation Aid per 50 µL PCR.

Quantitation and Assessment of RNA Purity by UV Absorbance

Quantitation of RNA by UV absorbance

The concentration of RNA can be determined by diluting an aliquot of the preparation (usually a 1:50 to 1:100 dilution) in TE (10 mM Tris-HCl pH 8, 1 mM EDTA), and reading the absorbance in a spectrophotometer at 260 nm. The concentration of RNA in µg/ml can be calculated as follows:

1 A_{280} = 40 µg RNA/mL

so, A_{260} × dilution factor × 40 = µg/mL RNA

For example:

An A_{260} value of 0.54 from a 180 µL RNA sample diluted 1:50 corresponds to an RNA concentration of:

0.54 × 50 × 40 = 1080 µg/mL or 1.08 mg/mL

The total yield of RNA is:

1.08 mg/mL × 0.18 mL = 0.19 mg

Be aware that any contaminating DNA in the RNA prep will lead to an overestimation of yield, since all nucleic acids absorb at 260 nm.

Assessing RNA purity by UV absorbance

The ratio of A_{260} to A_{280} provides an indication of protein contamination. For a relatively pure sample, the A_{260}/A_{280} ratio should fall in the range of 1.8 to 2.1. Even if an RNA preparation has an $A_{260/280}$ ratio slightly outside of this range it may function well in common applications such as Northern blotting, RT-PCR, poly(A) selection, and RNase protection assays.

Polysaccharide contamination of RNA preparations can be most easily assessed by determining the ratio of its absorbance at 260 nm to its absorbance at 230 nm. RNA preparations that are relatively free of polysaccharide contamination should have a ratio (A_{260}/A_{230}) of at least 2.

References

Baker SS, Clayton LR, Kamalay JC (1990) RNA and DNA Isolation from Recalcitrant Plant Tissues, *BioTechniques* 9(3):268–272.

Cheng S-H, Seeman JR (1998) Extraction and Purification of RNA from Plant Tissues Enriched in Polysaccharides. In: R Rapley and DL Manning (editors) *Methods in Molecular Biology, volume 86: RNA Isolation and Characterization Protocols*. Totowa, N.J: Humana Press, Inc. p 27–32.

Koonjul PK, Brandt WF, Farrant JM, Lindsey GG (1999) Inclusion of polyvinylpyrrolidone in the polymerase chain reaction reverses the inhibitory effects of polyphenolic contamination of RNA. *Nucleic Acid Res* 27:915–916.

Lopez-Gomez R, Gomez-lim MA (1992) A method for extracting intact RNA from fruits rich in polysaccharides using ripe mango mesocarp. *HortScience* 27:440–442.

Tesniere C, Vayda ME (1991) Method for the isolation of high-quality RNA from grape berry tissues without contaminating tannins or carbohydrates. *Plant Mole Biol Reporter* 9:242–251.

Wilkins TA, Smart LB (1996) Isolation of RNA from Plant Tissue. In: Krieg PA (editor) *A Laboratory Guide to RNA: Isolation, Analysis, and Synthesis* New York, NY:Wiley-Liss, Inc. p 21–42.

Quality Control

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

Nonspecific endonuclease activity

Meets or exceeds specification when a sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

Meets or exceeds specification when a sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

Appendix A Safety Information

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs), and use appropriate personal protective equipment (gloves, gowns, eye protection, etc.). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Depending on the samples used on the instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/ csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from:

- **www.invitrogen.com/sds**

  or

- **www.appliedbiosystems.com/sds**

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

- **www.invitrogen.com**

  or

- **www.appliedbiosystems.com**

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

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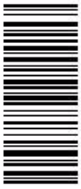

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