AG1 Lysis Buffer

Total Nucleic Acid Extraction
Catalog number: ACC 00760

LIST OF CONTENTS

Lot number	Item	
_____	ACC 00760/0060	60 mL
_____	Instructions	N/A

MATERIAL REQUIRED, BUT NOT PROVIDED

- Research grade Ethanol
- Research grade Isopropanol, 70%
- Micropipette
- Micropipette tips
- Microcentrifuge tubes
- Gloves
- Microcentrifuge
- Vortexer
- Sonicator (optional)
- Sample grinding device such as:
    - Agdia sample mesh bag (ACC 00930)
    - Agdia tissue homogenizer (ACC 00900)
- Mortar and pestle

INTENDED USE

The AG1 Lysis Buffer and Total Nucleic Acid Extraction protocol are used for the isolation of total RNA and DNA from plant materials.

This method is a fast and inexpensive alternative to commercial column-based extraction kits and does not require the use of phenol, chloroform, or β- mercaptoethanol. Nucleic acids isolated using this method may be used in a variety of downstream molecular biology applications, such as conventional PCR, RT-PCR, Real-time PCR, and dot blot hybridization.

This method is suitable for the recovery of viral RNA, viral DNA, bacterial DNA, and viroid RNA.

STORAGE

This buffer should be stored at room temperature (25°C) and used within 1 year of purchase.

Store bottle tightly closed in a dry and well-ventilated area. Do not store near acids.

PRECAUTIONS

Prevent direct skin and eye contact with, or ingestion of, product components. Obtain medical attention in case of accidental ingestion of kit components.

This buffer may cause skin and eye irritation. Disposable gloves and protective eyewear should be worn when handling this product. Please refer to Safety Data Sheet for more information.

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EXTRACTION PROCEDURE

Note: it is recommended that gloves be worn when taking tissue samples and performing the extraction procedure.

Prepare samples

- When possible, select leaf samples showing symptoms. In some cases, composites of up to ten leaves per sample can be used to make testing more economical. However, too many plant samples per composite can reduce test sensitivity. For recommendations on seed compositing, please contact us at techsupport@agdia.com.

- Grind plant tissue to produce a crude liquid extract (plant sap). You will need 50 µL of liquid per sample. For dry tissues, distilled water may be added to the sap in 50 µL increments in order to retrieve the required 50 µL for subsequent steps. When grinding samples, use Agdia's sample mesh bags (ACC 00930), Agdia's tissue homogenizer (ACC 00900), a mortar and pestle, or other grinding devices. If you are using a mortar and pestle, wash and rinse thoroughly between samples to avoid contamination.

Extract samples

- Add 50 µL of plant sap to a microcentrifuge tube containing 450 µL AG1 Lysis Buffer.

- Vortex the solution briefly. Incubate the microcentrifuge tube for 5 minutes at room temperature.

- Following incubation, centrifuge the tube at maximum speed (12000 x g or greater) for 3 minutes.

- Transfer 300 µL of supernatant to a new microcentrifuge tube containing 350 µL ethanol. Discard the original tube and pellet.

- Vortex the solution briefly to mix. Centrifuge the tube at maximum speed for 5 minutes to create a nucleic acid pellet.

- Discard the supernatant and wash the pellet twice with 300 µL of 70% isopropanol. Take care not to dislodge the pellet from the bottom or sides of the tube.

- Invert the tube on paper towel and allow to air dry for ≥ 10 minutes at room temperature.

- Resuspend the pellet in 40 to 50 µL nuclease-free water. Alternatively, 1 X TE buffer (10 mM TrisCl, pH 8.0; 1 mM EDTA, pH 8.0) may be used. To facilitate resuspension of the pellet, vortex the tube thoroughly. The tube may also be heated to 65°C and/or sonicated.

- Add 1 µL of RNase Inhibitor for long term storage of RNA extracts.

- Store the extracts at -20°C. For long term storage of RNA, -80°C storage is recommended.

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LIMITATIONS

The AG1 Lysis Buffer has been used with diverse tissue types in various molecular assays; however, the validation of every tissue type for every downstream application is not possible. Extracts using the AG1 Lysis Buffer have been known to produce a non-specific response on occasion. Please validate the use of the AG1 Lysis Buffer for your downstream application by using known negative and positive material to ensure that it is suitable for your use.

- This product is for research purposes only.
- This extraction method is validated for use in select Agdia products.
- This extraction method is recommended for plant tissues (leaves and seed) only. The quality and yield of nucleic acid produced from this method are dependent on the condition of the plant materials.

TECHNICAL SERVICE

If you have any questions about using this product, please contact Agdia, Inc. Monday-Friday by phone (574-264-2615 or 800-622-4342) or by email (techsupport@agdia.com). Visit our website www.agdia.com.