

EcoSpin Plant Total RNA Kit

Troubleshooting Guide

50 rxns

Cat No: E2096

Use this guide for common problems encountered during total RNA purification from plant material. For best results, follow the specified tissue input, homogenization, 60°C lysis, chloroform phase separation, careful upper-phase transfer, 70% ethanol addition, column loading, wash, dry-spin, and elution conditions in the product protocol.

Before You Start - Quick Checks

1. Store EcoSpin Lysis/Binding Buffer at 4-8°C upon receipt; store the remaining kit components at 15-25°C.
2. Use up to 300 mg plant material per preparation and weigh the sample before processing.
3. Homogenize thoroughly: grind in liquid nitrogen without allowing the tissue to thaw, or use a rotor-stator homogenizer at 15,000 rpm for 30 sec after adding 500 µL Lysis/Binding Buffer.
4. Incubate the lysate at 60°C for 5 min and keep the microcentrifuge tube cap tightly closed to prevent evaporation.
5. Add 100 µL chloroform, mix by shaking, incubate 3 min at room temperature, and centrifuge at 12,000 rpm for 15 min at 4°C.
6. Transfer only the upper phase to a new RNase-free tube; do not contaminate it with the lower yellow phase. Add an equal volume of 70% ethanol and mix well.
7. Load 700 µL per column spin. Add 40 mL absolute ethanol to EcoSpin Wash Buffer 2 concentrate before first use and complete the final 200 µL Wash Buffer 2 / 2 min spin.
8. Elute with 50-100 µL EcoSpin Elution Buffer applied to the center of the membrane; incubate 1 min at room temperature before centrifugation.

Problem	Most likely cause	Recommended action
Low or no RNA yield	Starting material exceeded 300 mg; plant tissue was not ground/homogenized completely; lysis conditions were shortened; the upper phase was incompletely recovered; or the equal-volume 70% ethanol step was not followed.	Use no more than 300 mg plant material per preparation. Homogenize thoroughly using liquid nitrogen or the rotor-stator method, add 500 µL EcoSpin Lysis/Binding Buffer, complete the 60°C / 5 min lysis step, recover the upper phase carefully after centrifugation, then add an equal volume of 70% ethanol before column loading.
Plant tissue is difficult to homogenize	Tissue was insufficiently ground, allowed to thaw during liquid-nitrogen processing, or not vigorously homogenized with the rotor-stator method.	For the liquid-nitrogen route, grind the tissue thoroughly and allow the liquid nitrogen to evaporate without allowing the plant tissue to thaw; immediately add 500 µL Lysis/Binding Buffer. Alternatively, homogenize vigorously at 15,000 rpm for 30 sec after adding 500 µL buffer.
Lysate remains heterogeneous after lysis	Mechanical disruption was incomplete or the 60°C lysis incubation was omitted or shortened.	Ensure complete grinding or rotor-stator homogenization first, then incubate the lysate at 60°C for the full 5 min. Mix the sample as specified before proceeding to chloroform extraction.
Sample volume decreases during the 60°C incubation	The microcentrifuge tube cap was not tightly closed, allowing evaporation of the Lysis/Binding Buffer.	Close the tube cap tightly during the 60°C / 5 min incubation, as specified in the protocol, to minimize evaporation and preserve the intended reagent ratio.
Phase separation is poor or the upper phase is difficult to identify	The 100 µL chloroform addition, 3 min room-temperature incubation, or 12,000 rpm / 15 min / 4°C centrifugation conditions were not followed exactly.	Add 100 µL chloroform, mix well by shaking, incubate for 3 min at room temperature, then centrifuge at 12,000 rpm for 15 min at 4°C before collecting the upper phase.
Upper phase is contaminated with lower yellow phase	The interface or lower yellow phase was disturbed during transfer after chloroform separation.	Transfer only the upper phase to a new RNase-free tube. Avoid aspirating material from the lower yellow phase or the interface during transfer.
Low recovery after upper-phase transfer	Too much upper phase was left behind to avoid the interface, or the equal-volume 70% ethanol addition was omitted or inaccurate.	Recover as much of the upper phase as possible without disturbing the lower phase. Measure or estimate the recovered upper-phase volume and add an equal volume of 70% ethanol; mix well before column loading.
Column flow is slow or liquid remains on the membrane	More than 700 µL was loaded at once, excessive plant material was used, or lower-phase/particulate material was transferred to the column.	Keep the starting material at or below 300 mg, transfer only the upper phase, add the specified equal volume of 70% ethanol, and load no more than 700 µL per spin. Repeat the loading step as necessary.
RNA purity is poor / downstream performance is weak	Lower yellow-phase carryover, incomplete washing, Wash Buffer 2 not prepared with ethanol, or residual wash buffer remained on the membrane.	Avoid lower-phase carryover. Add 40 mL absolute ethanol to the 10 mL EcoSpin Wash Buffer 2 concentrate before first use. Perform the 400 µL Wash Buffer 1, 500 µL Wash Buffer 2, and final 200 µL Wash Buffer 2 / 2 min maximum-speed steps completely.
Wash Buffer 2 gives inconsistent results	Absolute ethanol was not added to the Wash Buffer 2 concentrate before use.	Add 40 mL absolute ethanol to EcoSpin Wash Buffer 2 concentrate before first use and mix thoroughly. Use the prepared buffer consistently for both Wash Buffer 2 steps.

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Problem	Most likely cause	Recommended action
Ethanol / wash-buffer carryover in the eluate	The final 200 µL Wash Buffer 2 step or the 2 min maximum-speed centrifugation was omitted or shortened.	After the 500 µL Wash Buffer 2 wash, add 200 µL Wash Buffer 2 and centrifuge for the full 2 min at maximum speed. Then transfer the column to a clean RNase-free tube before elution.
Low RNA concentration in the eluate	A large elution volume was selected, Elution Buffer was not applied to the center of the membrane, or the 1 min incubation was skipped.	Apply 50-100 µL EcoSpin Elution Buffer directly to the center of the membrane and incubate for 1 min at room temperature before the 30 sec maximum-speed centrifugation. Use a consistent elution volume when comparing samples.
No or very little eluate is recovered	Elution Buffer did not adequately contact the membrane, the column was not transferred to a clean tube, or the final centrifugation was incomplete.	Transfer the column to a clean RNase-free 1.5 mL tube, add 50-100 µL Elution Buffer to the membrane center, incubate 1 min, and centrifuge 30 sec at maximum speed.
RNA integrity is poor or results vary between plant samples	Plant tissue warmed or thawed before lysis, homogenization was inconsistent, or sample handling and phase-transfer conditions differed between preparations.	Standardize tissue mass and the homogenization method. During liquid-nitrogen grinding, do not allow the tissue to thaw before adding Lysis/Binding Buffer. Keep the 60°C lysis, chloroform extraction, 4°C centrifugation, upper-phase transfer, ethanol addition, wash, and elution conditions identical between samples.
Results remain inconsistent despite correct reagent volumes	One or more timing, temperature, centrifugation, transfer, wash, or elution steps differed between runs.	Repeat the workflow using up to 300 mg tissue, 500 µL Lysis/Binding Buffer, 60°C for 5 min, 100 µL chloroform, 3 min at room temperature, 12,000 rpm for 15 min at 4°C, careful upper-phase transfer, equal-volume 70% ethanol, 700 µL loading portions, complete wash steps, and 50-100 µL elution.

Still having trouble? Re-check the starting tissue mass, complete homogenization, 500 µL Lysis/Binding Buffer, 60°C / 5 min lysis with a tightly closed cap, 100 µL chloroform addition, 3 min room-temperature incubation, 12,000 rpm / 15 min / 4°C phase separation, careful upper-phase transfer, equal-volume 70% ethanol addition, 700 µL loading portions, Wash Buffer 2 ethanol preparation, final 2 min wash spin, and 50-100 µL center-of-membrane elution. If the problem persists, contact EcoTech Biotechnology technical support with the plant material, starting mass, homogenization method, phase appearance, centrifugation conditions, and observed RNA yield or downstream issue.