

ClearBand TRUEzol Reagent

Troubleshooting Guide

100 mL

Cat No: TR100

Use this guide for common problems encountered during total RNA isolation with ClearBand TRUEzol Reagent and during optional recovery of DNA and protein from the remaining phases. Follow the starting-material limits, homogenization instructions, phase-separation conditions, precipitation steps, wash conditions, and recovery procedures in the product protocol.

Before You Start - Quick Checks

1. Use 1 mL TRUEzol Reagent for up to 1×10^7 cells or about 100 mg tissue; for tissue, homogenize 50-100 mg in 1 mL reagent.
2. For monolayer cells, use 1 mL reagent per 10 cm² culture area. For suspension cells, pellet at 200 x g for 5 minutes before lysis.
3. After homogenization, incubate 5 minutes at room temperature, then add 0.2 mL chloroform per 1 mL TRUEzol Reagent.
4. Shake for 15 seconds, incubate 3 minutes at room temperature, and centrifuge at 12000 x g for 15 minutes at 4°C.
5. Transfer only the clear upper aqueous phase for RNA isolation; avoid the interphase and pink lower organic phase.
6. Precipitate RNA with 0.5 mL isopropyl alcohol per 1 mL TRUEzol used, then centrifuge at 12000 x g for 10 minutes at 4°C.
7. Wash RNA with 1 mL 75% ethanol per 1 mL reagent used and centrifuge at 7500 x g for 5 minutes at 4°C.
8. If DNA or protein recovery is planned, retain the interphase and organic/phenol-ethanol fractions and follow their specific protocols.

Application	Problem	Most likely cause	Recommended action
RNA	Low or inconsistent RNA yield	Too much starting material was used for the amount of TRUEzol Reagent, or the sample was not homogenized/lysed completely.	Use 1 mL TRUEzol Reagent for up to 1×10^7 cells or about 100 mg tissue. For tissue, homogenize 50-100 mg in 1 mL reagent; for cultured cells, follow the specified culture-area or pellet workflow.
RNA	High-fat tissue gives poor phase separation or low purity	Lipids remain in the homogenate and interfere with clean phase separation.	After homogenizing high-fat tissue, centrifuge for 5 minutes at 12000 x g and 4°C and remove the upper fat layer before continuing.
RNA	Plant homogenate contains excessive debris	Insoluble plant material was carried into the phase-separation step.	After plant homogenization, centrifuge at 12000 x g for 10 minutes at 4°C. Transfer only the cleared homogenate to a new RNase-free tube before proceeding.
RNA	Incomplete lysis of monolayer cells	Insufficient TRUEzol volume was used for the culture surface area or the lysate was not pipetted enough to disrupt cells.	Remove culture medium and lyse directly in the plate/flask using 1 mL TRUEzol Reagent per 10 cm ² area. Pipette the lysate several times to ensure complete disruption.
RNA	Low recovery from suspension cells	Cells were not pelleted efficiently or part of the pellet was lost before lysis.	Pellet suspension cells at 200 x g for 5 minutes at room temperature, remove the medium carefully, then lyse the pellet in 1 mL TRUEzol Reagent and pipette thoroughly.
RNA	Poor or unclear phase separation	Chloroform amount, shaking, incubation, centrifugation force, temperature, or duration did not match the protocol.	After 5 minutes at room temperature, add 0.2 mL chloroform per 1 mL TRUEzol used. Shake for 15 seconds, incubate 3 minutes, and centrifuge at 12000 x g for 15 minutes at 4°C.
RNA	Aqueous phase is pink or visibly contaminated	Interphase or lower organic phase was disturbed during transfer of the upper aqueous phase.	Transfer only the clear upper aqueous phase into a new RNase-free tube. Avoid aspirating material from the interphase or pink lower organic phase.
RNA	Low or invisible RNA pellet	Isopropanol volume, precipitation time, or centrifugation conditions were incorrect, or starting material was insufficient.	Add 0.5 mL isopropyl alcohol per 1 mL TRUEzol Reagent used, incubate for 10 minutes at room temperature, and centrifuge at 12000 x g for 10 minutes at 4°C.
RNA	Poor RNA purity / downstream inhibition	Organic/interphase carryover occurred or the 75% ethanol wash was incomplete.	Perform a clean aqueous-phase transfer. Wash the RNA pellet with 1 mL 75% ethanol per 1 mL TRUEzol used, vortex, and centrifuge at 7500 x g for 5 minutes at 4°C.
RNA	RNA pellet is lost during wash	The pellet is small and was aspirated together with the supernatant.	Remove supernatant carefully with a micropipette after precipitation and washing. Keep tube orientation consistent so the pellet location remains easy to track.
RNA	RNA pellet does not dissolve readily	The air-dried pellet has not completely re-dissolved in DEPC-treated water.	Dissolve by pipetting up and down in DEPC-treated water. If necessary, incubate for 10 minutes at 55-60°C before storage.
RNA	RNA quality decreases during delayed processing	The homogenized sample was stored outside the conditions stated in the protocol.	Process promptly or store homogenized samples at 4°C overnight or at -70°C for up to one year, as specified in the datasheet.
DNA	Low DNA recovery from the interphase	Residual aqueous phase remained over the interphase, or ethanol precipitation conditions were incorrect.	Remove residual aqueous phase, add 0.3 mL absolute ethanol per 1 mL TRUEzol used, mix by inversion, incubate 2-3 minutes, and centrifuge for 5 minutes at 2000 x g and 4°C.

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Application	Problem	Most likely cause	Recommended action
DNA	DNA pellet contains contaminants	The sodium citrate/ethanol wash sequence was incomplete.	Wash with 1 mL of 0.1 M sodium citrate in 10% ethanol, pH 8.5, per 1 mL TRUEzol used. Incubate 30 minutes with occasional gentle inversion, centrifuge at 2000 x g for 5 minutes at 4°C, and repeat once.
DNA	DNA remains difficult to resuspend	Isolated DNA does not resuspend efficiently in water or Tris buffer.	After the 75% ethanol wash and 5-10 minute drying step, resuspend the pellet in 0.3-0.6 mL of 8 mM NaOH by pipetting up and down.
DNA	Insoluble material remains in the DNA preparation	Debris remains after resuspension in 8 mM NaOH.	Centrifuge for 10 minutes at 12000 x g and 4°C and transfer the clarified supernatant to a new tube.
DNA	DNA storage performance is poor	Long-term storage solution was not adjusted to the recommended pH or EDTA concentration.	For longer-term storage at -20°C, adjust the solution to pH 7-8 with HEPES and add EDTA to 1 mM. Overnight storage may be performed at 4°C.
Protein	Low or no protein recovery	The phenol-ethanol supernatant generated during DNA precipitation was discarded, or the protein precipitation ratio was incorrect.	Retain the phenol-ethanol supernatant after DNA pelleting. Add 1.5 mL isopropanol per 1 mL TRUEzol Reagent used, incubate 10 minutes, and centrifuge 10 minutes at 12000 x g and 4°C.
Protein	Protein pellet remains contaminated	The guanidine hydrochloride/ethanol wash sequence was incomplete.	Use 0.3 M guanidine hydrochloride in 95% ethanol. Resuspend the pellet in 2 mL wash solution per 1 mL TRUEzol used, incubate 20 minutes, centrifuge at 7500 x g for 5 minutes at 4°C, and repeat the wash twice.
Protein	Protein pellet is difficult to dissolve	The air-dried pellet has not fully resuspended in the final SDS solution.	Resuspend the pellet in 200 µL of 1% SDS by pipetting up and down. If needed, incubate at 50°C in a water bath or heat block to promote complete resuspension.
Protein	Insoluble material remains after protein resuspension	Insoluble material remains in the 1% SDS suspension.	Centrifuge for 10 minutes at 10000 x g and 4°C, then transfer the clarified supernatant to a new tube before downstream measurement or use.
Protein	Protein wash/storage conditions are inconsistent	The pellet was not kept in the specified guanidine hydrochloride/ethanol wash solution or storage conditions.	The datasheet states that proteins can be stored in the wash solution for at least 1 month at 4°C or at least 1 year at -20°C.

Still having trouble? Re-check the starting-material-to-TRUEzol ratio, sample homogenization, phase-separation centrifugation, clean transfer of the aqueous phase, precipitation reagent volumes, and wash steps. For optional DNA or protein recovery, confirm that the correct interphase or phenol-ethanol fraction was retained. If the problem persists, contact EcoTech Biotechnology technical support with sample type and amount, TRUEzol volume, centrifugation conditions, phase appearance, and observed RNA/DNA/protein yield or purity.