

ClearBand TRUEzol LS Reagent

Troubleshooting Guide

100 mL

Cat No: TRLS100

Use this guide for common problems encountered during total RNA isolation with ClearBand TRUEzol LS Reagent and during optional recovery of DNA and protein from the remaining phases. Follow the reagent-to-sample ratios, phase-separation conditions, precipitation steps, wash conditions, and recovery instructions in the product protocol.

Before You Start - Quick Checks

1. TRUEzol LS Reagent is intended for liquid biological materials and is not suitable for whole blood.
2. Keep the TRUEzol LS-to-sample ratio at 3:1: use 0.75 mL reagent for 0.25 mL sample; bring smaller samples to 0.25 mL with water first.
3. For phase separation, add 0.2 mL chloroform per 0.75 mL TRUEzol LS, shake 15 seconds, incubate 3-15 minutes, and centrifuge at 12000 x g for 15 minutes at 4°C.
4. Transfer only the clear upper aqueous phase for RNA isolation; avoid the interphase and pink lower organic phase.
5. Precipitate RNA with 0.5 mL isopropyl alcohol per 0.75 mL TRUEzol LS used, then centrifuge at 12000 x g for 10 minutes at 4°C.
6. Wash the RNA pellet with 1 mL 75% ethanol per 0.75 mL reagent used and centrifuge at 7500 x g for 5 minutes at 4°C.
7. If DNA or protein recovery is planned, retain the interphase and organic phase and follow their specific downstream protocols.

Application	Problem	Most likely cause	Recommended action
RNA	Low or inconsistent RNA yield	The TRUEzol LS-to-sample ratio was not kept at 3:1, or a sample smaller than 0.25 mL was processed without first adjusting the sample volume to 0.25 mL.	Use 0.75 mL TRUEzol LS Reagent for 0.25 mL liquid sample. If sample volume is below 0.25 mL, adjust it to 0.25 mL with water before adding reagent. Keep the reagent-to-sample volume ratio at 3:1.
RNA	Poor lysis or incomplete homogenization	The liquid sample was not mixed sufficiently with TRUEzol LS Reagent before phase separation.	Mix 0.75 mL TRUEzol LS with 0.25 mL sample and homogenize by passing the suspension several times through a pipette. Incubate the homogenized sample for 5 minutes at room temperature before chloroform addition.
RNA	Whole-blood sample gives poor or unreliable results	TRUEzol LS Reagent is specified for liquid biological materials but is not suitable for whole blood.	Do not use the TRUEzol LS protocol for whole blood. Use this reagent only with the liquid biological materials supported by the product datasheet.
RNA	Poor or unclear phase separation	Chloroform amount, mixing, incubation time, centrifugation force, temperature, or duration did not match the protocol.	Add 0.2 mL chloroform per 0.75 mL TRUEzol LS used, shake thoroughly for 15 seconds, incubate 3-15 minutes at room temperature, then centrifuge at 12000 x g for 15 minutes at 4°C.
RNA	Aqueous phase is pink or visibly contaminated	The interphase or lower organic phase was disturbed during transfer of the upper aqueous phase.	Transfer only the clear upper aqueous phase to a new RNase-free tube. Avoid disturbing or aspirating material from the interphase and pink lower organic phase.
RNA	RNA purity is poor / downstream reactions are inhibited	Interphase or organic-phase carryover occurred during aqueous-phase transfer, or the RNA wash was incomplete.	Repeat the workflow with a clean aqueous-phase transfer. Wash the RNA pellet with 1 mL 75% ethanol per 0.75 mL TRUEzol reagent used, vortex, and centrifuge at 7500 x g for 5 minutes at 4°C.
RNA	RNA pellet is very small or not visible	The isopropanol amount, precipitation time, centrifugation conditions, or starting sample/reagent ratio was incorrect.	Add 0.5 mL isopropyl alcohol per 0.75 mL TRUEzol LS Reagent used, incubate 10 minutes at room temperature, then centrifuge at 12000 x g for 10 minutes at 4°C. Re-check the 3:1 reagent-to-sample ratio.
RNA	RNA pellet is lost during processing	The pellet is small and was removed together with the supernatant after precipitation or washing.	Remove supernatant carefully with a micropipette after the precipitation and wash centrifugations. Keep the tube orientation consistent so the pellet location is easy to track.
RNA	RNA pellet does not re-dissolve readily	The RNA pellet has not completely dissolved in DEPC-treated water after air-drying.	Dissolve the air-dried pellet in DEPC-treated water by pipetting up and down. If necessary, incubate for 10 minutes at 55-60°C before storage.
RNA	RNA quality decreases before phase separation	The homogenized sample was stored beyond the stated pre-processing conditions.	After homogenization, process promptly or store the homogenate only under the specified conditions: 4°C overnight or -70°C for up to one month.
RNA	Unexpected contamination after RNA isolation	The interphase and organic phase were not kept separate from the RNA-containing aqueous phase.	During phase separation, identify the colorless upper aqueous phase clearly and transfer it alone. Keep the interphase and organic phase separately at 2-8°C only if DNA or protein recovery is planned.
DNA	Low DNA recovery from the interphase	Residual aqueous phase was left over the interphase, the ethanol ratio was incorrect, or the DNA precipitation spin was not performed as specified.	Remove residual aqueous phase. Add 0.3 mL absolute ethanol per 0.75 mL TRUEzol LS used, mix by inversion, incubate 2-3 minutes, and centrifuge 5 minutes at 2000 x g and 4°C.

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Application	Problem	Most likely cause	Recommended action
DNA	DNA pellet contains impurities	The sodium citrate/ethanol washes were incomplete or performed only once.	Wash with 1 mL 0.1 M sodium citrate in 10% ethanol, pH 8.5, per 0.75 mL TRUEzol reagent used. Incubate 30 minutes with occasional gentle inversion, centrifuge 5 minutes at 2000 x g and 4°C, then repeat the wash once.
DNA	DNA is difficult to resuspend	The isolated DNA pellet does not resuspend well in water or Tris buffer.	After the 75% ethanol wash and 5-10 minute drying step, resuspend the DNA pellet in 0.3-0.6 mL of 8 mM NaOH by pipetting up and down, as specified in the datasheet.
DNA	Insoluble material remains after DNA resuspension	Insoluble material remains in the 8 mM NaOH DNA suspension.	Centrifuge for 10 minutes at 12000 x g and 4°C, then transfer the clear supernatant to a new tube.
DNA	DNA is unstable during longer-term storage	The pH was not adjusted to 7-8 and EDTA was not added before long-term storage at -20°C.	For longer-term storage at -20°C, adjust the DNA solution to pH 7-8 with HEPES and add EDTA to 1 mM. For overnight storage, the datasheet specifies 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 LS used, incubate 10 minutes at room temperature, and centrifuge 10 minutes at 12000 x g and 4°C.
Protein	Protein pellet remains contaminated after precipitation	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 0.75 mL TRUEzol LS used, incubate 20 minutes, centrifuge 5 minutes at 7500 x g and 4°C, and repeat the wash two additional times.
Protein	Protein pellet is difficult to dissolve	The air-dried protein 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 the sample at 50°C in a water bath or heat block to promote complete resuspension.
Protein	Insoluble material remains in the protein preparation	Insoluble material remains after resuspension in 1% SDS.	Centrifuge for 10 minutes at 10000 x g and 4°C, then transfer the clarified supernatant to a new tube before protein measurement or downstream use.
Protein	Protein wash/storage conditions are inconsistent	The protein pellet was not maintained in the specified wash solution or storage conditions.	The datasheet states that proteins can be stored in the guanidine hydrochloride/ethanol wash solution for at least 1 month at 4°C or at least 1 year at -20°C.

Still having trouble? Re-check the 3:1 TRUEzol LS-to-sample ratio, phase-separation centrifugation, clean transfer of the aqueous phase, precipitation reagent volumes, and the specified 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 volume, TRUEzol LS volume, centrifugation conditions, phase appearance, and observed RNA/DNA/protein yield or purity.