

Troubleshooting: RNA Isolation with TRIzol Reagent

Problem	Possible Cause	Suggested Solution
Low yield of RNA	RNA not solubilized completely.	To increase solubilization, pipet RNA pellet repeatedly in SDS or DEPC-treated water. Heat sample at 55°C for 10 to 15 min. Do not allow RNA pellet to dry completely. Do not lyophilize or vacuum dry samples. Note: Clear pellet indicates over-drying. Do not centrifuge RNA above 12,000×g.
	Sample not homogenized completely.	Make sure no particulate matter remains. Be sure to incubate for 5 min at room temperature after homogenization.
Degraded RNA	Sample manipulated too much before TRIzol Reagent addition.	Process tissue immediately after removal from animal. For cell culture samples, minimize washing steps. Add TRIzol Reagent directly to plates. Do not trypsinize cells.
	Improper storage of RNA.	Store isolated RNA at -70°C, not -20°C.
	Frozen tissue thawed in absence of TRIzol Reagent.	Add frozen tissue to TRIzol Reagent.
Low A _{260/280} (< 1.65)	Residual organic solvents in the RNA (phenol, chloroform).	Be sure not to carry any of the organic phase with the RNA sample. Precipitate the RNA again with ethanol.
	Sample not homogenized with sufficient TRIzol Reagent.	Use 1 ml TRIzol Reagent for up to 50 mg tissue or 10 ⁶ cells. Be sure to incubate sample for 5 min at room temperature after homogenization.
	pH of solution is acidic.	Dissolve sample in TE instead of water.
	A ₂₆₀ or A ₂₈₀ outside the linear range.	Dilute sample to bring absorbance into linear range.
RNA contains some DNA	Part of the interphase was removed with the aqueous phase.	Be sure not to take any of the interphase (contains the DNA) with the aqueous phase.
	Insufficient TRIzol Reagent used.	Use 1 ml of TRIzol Reagent for 50 mg of tissue or 10 ⁶ cells.
	Cells or tissue contained organic solvents.	Be sure original sample does not contain organic solvents such as ethanol or DMSO.
	Insoluble materials were not removed before chloroform extraction.	Remove any particulate material before chloroform addition. This material may trap DNA.

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Aqueous phase containing RNA has color	For tissues with high fat content (<i>i.e.</i> skin), fat micelles do not completely separate to top of aqueous phase during centrifugation. Micelles pick up red color from TRIzol Reagent.	Centrifuge sample before chloroform addition and remove fat layer on top.
	For samples with high amounts of blood, some iron or hemoglobin may remain in aqueous phase giving a yellowish or maroon color.	Make sure not at the upper range of sample to TRIzol Reagent ratio.
	Aqueous phase turns yellow upon addition of isopropanol.	Try a fresh bottle of isopropanol. (Note: this color has not inhibited RT-PCR).
Cells don't detach from flask after TRIzol Reagent addition.	This can be seen with some strongly adherent cells.	After TRIzol Reagent addition, let cells sit 2 to 3 min. Scrape cells with a rubber policeman. Incubate several minutes. Collect cells and repeatedly pipet cells over flask surface. Then transfer homogenate to a tube.
Precipitate in bottom of the tube after chloroform addition and centrifugation.	High amounts of polysaccharides or proteoglycans.	Remove aqueous phase and alter isopropanol precipitation step to using 0.25 volumes of 1.2 M sodium citrate, 0.8 M NaCl, and 0.25 volumes of isopropanol.