Troubleshooting Guide

Problem	Possible Reason	Solution
Little or no RNA eluted	**Insufficient disruption or homogenization**	Reduce the amount of starting sample and perform more disruption and homogenization. Incomplete lysis of the starting material will reduce RNA yields below expectations even if the binding capacity of the RNA Mini Column is not exceeded.
	Clogged Total RNA column because of excessive starting material	Reduce the amount of starting material and increase disruption and homogenization measures. Centrifuge the lysate to remove insoluble materials and use the supernatant only. It is essential to use the correct amount of starting material in order to obtain optimal RNA yield and purity. If the binding capacity of the RNA Mini Column is exceeded, RNA yields will be negatively affected in terms of quantity and reproducibility. Do not overload the RNA Mini Column, as this will significantly reduce RNA yield and purity!
	RNA is degraded	Starting sample should be fresh or frozen in liquid nitrogen. Storage at –80 °C is essential. Improper handling of the sample or storing the sample at –20 °C will cause RNA degradation. It is important that tissue samples are immediately frozen in liquid nitrogen and stored at -80°C. See also general remarks on RNA handling under "**VI. Working with RNA – Some Handling Guidelines"** in the manual.

Troubleshooting Guide

Problem	Possible Reason	Solution
Little or no RNA eluted	**RNase contamination**	Use RNase-free liquid handling tips and tubes. See also general remarks on RNA handling under "**VI. Working with RNA – Some Handling Guidelines"** in the manual.
DNA contamination		Refer to Protocol V for "DNAse Removal of Genomic DNA from eluted total RNA - phenol/chloroform" or other suitable DNAse removal procedures/reagents. For analysis of very low –abundance targets, any interference by residual DNA contamination can be detected by performing real-time PCR control experiments in which no reverse transcriptase is added prior to the PCR step.
A260/A280 ratio of eluted total RNA is low	**ddH_2O with a pH<7 was used to dilute RNA sample for spectrophotometric analysis**	Use 10 mM Tris-HCl of pH 7.5 or TE buffer to dilute RNA sample. The A260/A280 ratio is influenced considerably by pH. Lower pH results in a lower A260 / A280 ratio and reduced sensitivity to protein contamination.
	DNA is co-purified with RNA	Refer to Protocol V for "DNAse Removal of Genomic DNA from eluted total RNA - phenol/chloroform" or other suitable DNAse removal procedures/reagents.