Troubleshooting Guide –

NOVA Blood RNA Extraction kit

NOVA Tissue and Mammalian Cell Culture RNA Extraction Kit

NOVA Wilozole RNA Extraction kit

The following guide can be used to troubleshoot any RNA extractions using the *NOVA* kits.

If you require any additional information, please email us at info@willowfort.co.uk.

Problem	Causes	Possible Actions
Genomic DNA within RNA sample	Insufficient degradation of DNA during lysis	• Treat samples with DNase, but make sure DNase is removed after the reaction is complete
	Silica spin columns overloaded	• Follow the sample size suggestions to prevent overload
Low RNA yield	Starting sample was too small	• Use the suggested starting material • Do not overload the spin columns
	Cells were not lysed thoroughly	• Optimise the lysis step, particularly samples with tough structures
	Sample was not stored correctly	• Endogenous RNase may have degraded RNA. Adding chaotropic agents will inactivate any RNase present.
	RNase degraded RNA sample during or after extraction	• Ensure the correct protective clothing are used during extraction • Make sure consumables are RNase free • Prepare an RNase free environment by cleaning surround area with 70% ethanol and dH_2O
Low RNA integrity	Sample was not stored correctly	• Endogenous RNase may have degraded RNA. Adding chaotropic agents will inactivate any RNase present.
	RNase degraded RNA sample during or after extraction	• Ensure the correct protective clothing are used during extraction • Make sure consumables are RNase free • Prepare an RNase free environment by cleaning surround area with 70% ethanol and dH_2O
	Extracted total RNA was not stored correctly	• It is advised that due to its instability, RNA should be stored at -80°C to reduce risk of degradation.

Problem	Causes	Possible Actions
Inhibitors in RNA sample	A_{260}/A_{280} is < 1.7, therefore proteins or chaotropic agents are present.	• Avoid overloading the silica-based columns. • If your sample has high concentrations of protein, treatment of proteases is recommended before purification. • Follow washing steps closely to prevent carryover of contaminants
	A_{260}/A_{230} is < 1.0	• Follow washing steps closely to reduce risk of salt carryover. Increase number of washes if necessary. • Wash sample in ethanol to remove salts.
Problems with downstream applications	Poor quality RNA	• RNases may have degraded your DNA sample. • Store all RNA samples at -80ºC or less.
	Presence of contaminants	• Avoid overloading the silica-based columns. • If your sample has high concentrations of protein, treatment of proteases is recommended before purification. • Follow washing steps closely to prevent carryover of contaminants • Repeat RNA precipitation with salts and ethanol.
	Presence of residual ethanol	• Ensure silica filters have been thoroughly dried before eluting sample.