Troubleshooting Guide –

NOVA Blood DNA Extraction kit

NOVA Tissue and Mammalian Cell Culture DNA Extraction Kit

NOVA Viral RNA and DNA Extraction kit

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

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

Problem	Causes	Possible Actions
DNA is poor quality	Starting material was not stored correctly	• Nucleases may have degraded your DNA sample. • Store all DNA samples at -20ºC or less. • Do not store DNA in water for long periods of time. Instead use TE buffer. • DNA may have been fragmented, making it impossible to use for studies requiring high-molecular-weight DNA applications.
	Genomic DNA was not stored correctly	
	DNA was extracted from fixed tissue	
DNA yield is poor	Starting sample was too small	• Try using a large volume/weight of sample. • If a large volume/weight is not possible, amplification may be required. • Nucleases may have degraded your DNA sample.
	Cells were not lysed thoroughly	• Optimise the lysis step, particularly samples
	Poor DNA extraction	• Proteinase K may have been made too soon. Make the proteinase K solution up to 1 hour before extraction. • Ensure samples are well mixed with the lysis buffer. Vortex and pipette, if necessary.
DNA sample is cloudy	Transfer of cellular debris with DNA sample	• Centrifuge sample again, taking care to remove only the DNA sample
	Cellular debris not separate from DNA sample	• Centrifuge at a high speed, or for a longer time if high speeds are not possible.
The A_{260}/A_{280} ratio does fall within the recommended range	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_{280} is > 1.9, therefore RNA could be present.	• Treat samples with RNase, if samples have high concentrations of RNA. Some applications are not affected by RNA.

Problem	Causes	Possible Actions
Problems with downstream applications	Poor quality DNA	• Nucleases may have degraded your DNA sample. • Store all DNA samples at -20ºC or less. • Do not store DNA in water for long periods of time. Instead use TE buffer. • DNA may have been fragmented, making it impossible to use for studies requiring high-molecular-weight DNA applications.
	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 DNA precipitation with salts and ethanol.
	Presence of residual ethanol	• Ensure silica filters have been thoroughly dried before eluting sample.