Troubleshooting Guide –

HERA qPCR Kit

HERA SYBR® Green qPCR kit

HERA qPCR Kit wit UDG

HERA SYBR® Green qPCR kit with UDG

HERA^{PLUS} qPCR Kit

HERA^{PLUS} SYBR® Green qPCR kit

The following guide can be used to troubleshoot any reactions using the *HERA* qPCR kits.

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

Problem	Causes	Possible Actions
No or low Amplification	Poor DNA purity	• Consider sample specific purification kits for DNA • Measure DNA purity using a UV spectrophotometer. • Remove salts and inhibitors by repeating purification.
	Low quantity of DNA/degraded template	• Check DNA concentrations using a UV spectrophotometer. • Use freshly extracted DNA to ensure suitable amplification. • Reduce the freeze-thaw cycles, to reduce degradation.
	Inhibitor Present	• Dilute the DNA template to reduce inhibitors present • Repeat DNA extraction, taking care to minimise the carryover of inhibitors.
	Probe quality	• Reduce the number of freeze-thaw cycles by aliquoting into fresh nuclease-free eppendorfs • Do not store diluted probes • Only store hybridization probes at -20ºC
	Insufficient time and temperature for the amplification reaction	• Check qPCR machine for correct time and temperature • Optimise the thermal cycling conditions e.g. allow more time for genomic DNA to allow sufficient degradation and extension.
	Reagent quality	• Refer to manufacturer's storage suggestions • Only use fresh reagents to ensure suitable amplification • Reduce the number of freeze-thaw cycles • Ensure a complete mix of reagents • Check for any precipitation in any reagents.

Problem	Causes	Possible Actions
Higher Ct value than expected	DNA template is damaged or degraded	• Review DNA extraction protocol
	DNase contamination	• Ensure a clean work area. Use 70% ethanol and dH_2O to clean all equipment and space.
	Inhibitors present	• Use an additional wash of ethanol to remove inhibitors during DNA extraction. • Review DNA extraction protocol
Low Ct value than expected or lower Ct value than positive control	Template contamination	• Identify source of contamination by checking and replacing reagents. • Use separate pipettes for pre- and post-PCR reactions. • Ensure a clean work area. Use 70% ethanol and dH_2O to clean all equipment and space
	Concentration of DNA sample are too high	• Confirm the DNA concentration. • Decrease concentrations of DNA in PCR set-up
PCR Reaction Not Reproducible or Reaction Stopped Working	Error in cycling conditions	• Use the same thermal cycler for optimization and all future experiments. • Some primers are sensitive to temperature change. Double check primer specifications.
	dNTPs, primers or sample degraded	• Aliquot components to reduce number of freeze-thaw cycle.
	Error in set up	• Check correct pipetting technique • Create a master mix of all reagents other than DNA template. Aliquot this into separate PCR tubes. • Pipette DNA template into master mix • Ensure correct reagents have been added • Check correct thermal cycler program has been selected.
	Change in component	• Check for new components added to the master mix (e.g. new batch of primers)
	Inhibitors in template	• Decrease template concentration. • Repeat DNA extraction, taking care to minimise the carryover of inhibitors.

Problem	Causes	Possible Actions
Non-Specific Amplification	DNA contamination	• Use a control e.g. no-template control • Ensure good laboratory practice and sterile conditions.
	Error with primer design	• Increase primer length to increase specific amplification. • Increase annealing temperature to improve specificity of primer binding. • Check primer design in BLAST to ensure correct sequence
Amplification in Negative control	Contamination in DNA template sample	• Repeat reaction to check samples • Make PCR specific work areas to minimise pre- and post- contamination. • Ensure good laboratory practice and sterile conditions.
	Primer-dimer formation	• Primer concentration maybe too concentrated. Try reducing concentrations of forward and reverse primers • Perform a melt-curve to check the presence of primer-dimers • Re-evaluate the primer design, double checking for complementary structures.