

Summary of QRT-PCR detection strategy

ABgene® Verso 1-Step & 2-Step QRT-PCR Kits

	Advantages	Disadvantages
1-step QRT-PCR	<p>The QPCR reaction immediately follows the RT step which means results are obtained faster</p> <p>No manual intervention is required between the RT and QPCR steps which can improve reproducibility</p> <p>The reaction vessel is not opened between the RT and QPCR steps thereby, minimizing the risk of contamination</p> <p>The entire RT reaction is used in the QPCR reaction potentially giving improved detection of low copy number templates</p>	<p>The entire RT reaction is consumed in the subsequent QPCR making 1-step a more expensive strategy than 2-step QRT-PCR</p> <p>The complete consumption of the RT reaction means it cannot be used for quantifying additional targets</p> <p>The buffering conditions for 1-step QRT-PCR are suitable but not ideal for either phase making optimization difficult</p> <p>Random primers cannot be used during the RT step as this leads to non-specific amplification during the QPCR step</p>
2-step QRT-PCR	<p>The completed RT reaction can be aliquoted and used in multiple QPCR experiments</p> <p>The RT and QPCR steps can be individually optimized to give maximum assay performance</p> <p>The separation of the RT and QPCR steps allow a more flexible priming strategy ie. random primers and anchored oligo dTs are permissible in the RT step</p>	<p>2-step QRT-PCR takes longer to complete and requires more manual handling which increases the likelihood of human error</p> <p>Transferring an aliquot of the completed RT reaction to the QPCR vessel increases the risk of contamination</p> <p>The RT and QPCR steps are carried out in different reaction vessels which increases the volume of plastic waste</p>