# One-step real-time RT-PCR versus two-step real-time RT-PCR

**Real-time PCR has become an increasingly popular technique for analysis of gene expression. There are two primary methods of real-time PCR that can be performed. The first involves including the reverse transcriptase step in the same tube as the PCR reaction (one-step). The second method involves creating cDNA first by means of a separate reverse transcription reaction and then adding the cDNA to the PCR (two-step). There are advantages and disadvantages to both systems that you should considered before choosing the best one for your application.**

| | One-Step real-time RT-PCR | Two-step real-time RT-PCR |
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| Description | • RT and real-time PCR performed in the same tube | • RT and real-time PCR performed in separate tubes |
| Pros | • Less optimization required<br>• Simple and rapid<br>• Fewer pipetting steps (reducing possible errors and contamination)<br>• No contamination between RT and real-time PCR steps<br>• Best option for high-throughput screening (less time consuming than two-step reactions)<br>• Best method when only a few assays are run repeatedly | • Two buffers optimized for independent RT and real-time PCR<br>• Highly sensitive<br>• Potentially more efficient because random primers and oligo d(T) can be used<br>• Possibility to stock cDNA to quantify several targets<br>• Recommended when the reaction is performed with a limiting amount of starting material. |
| Cons | • Usually less sensitive than a two-step assay as the buffer is a compromise between the RT and real-time PCR buffers<br>• Difficult to troubleshoot RT step<br>• No stock of cDNA | • Time consuming<br>• More pipetting steps (increases possible error and contamination)<br>• Requires more optimization |

When performing reverse transcription reaction for a two-step assay, it is possible to use three different types of primers. Random Hexamer primers, which bind anywhere on the RNA and allow full coverage of total RNA (including ribosomal, bacterial and viral RNA), oligo $d(T)_{18}$, which binds to the poly-A tail of the mRNA which is located at the 3'-end of eukaryotic transcripts leading to full transcripts and specific primers, which bind to the transcript of interest.

When performing reverse transcription reaction for a one-step assay, only specific primers can be used, which bind to the transcript of interest.

SensiFAST One-Step kits therefore offer a quick and simple method to detect mRNA and so are useful when analyzing a few genes over a large number of samples. Since both the RT and PCR occur in the same tube, there is less pipetting and sample manipulation, possibly reducing variation and potential contamination. SensiFAST One-Step kits however, require the use of gene specific primers. Also, the reaction conditions needed to support both the RT and PCR may not be optimal for either reaction. Another drawback is that it is not possible to archive the cDNA produced during the reverse transcription reaction.

In contrast two-step real-time RT-PCR, using Tetro cDNA Synthesis kit and SensiFAST kit, offers a truly accurate determination of mRNA and is useful when analyzing a large number of transcripts over a few samples. Tetro cDNA Synthesis kit has flexibility in the priming strategy, allowing for oligo-dT, random primers or gene specific primers and is generally more sensitive than one-step as the RT and PCR occur separately and can be optimized individually. Also, the cDNA produced is more stable than the initial RNA sample and can be more easily archived for future use.