

RT qPCR without prior execution of a reverse transcription

RT qPCR for amplification of specific tRNA directly from RNA using a general forward primer and a specific reverse primer:

Performing the analysis in specific white qPCR plates

1. Set up reaction as follows:

Table 1: Representation of the composition for RT qPCR without prior reverse transcription

Component	reaction
HotScriptase (genaxxon)	5 µL
Primer forward (10pmol/µl)	0,8 µL
specific tRNA Primer (10pmol/µl)	0,8 µL
H2O	2,9 µL
ligated tRNA (8ng/µl)	0,5 µl

For the negative control 4,5 µl of H2O are used instead of above-mentioned primers and H2O.

2. Executing the following program in the qPCR Tower (qTOWER³, Analytik Jena)

Table 2: Representation of temperatures, times and cycle numbers for qPCR without prior reverse transcription

Period of time	temperature	number of cycles
3 min	95 °C	
15 s	95 °C	
30 s	59,5 °C	
45 s	68 °C	45x