qACE™ Fast Probe qRT-PCR 4X Supermix

#GE55.0200 (1 ml) | GE55.5200 (5x 1ml) | GE55s (trial size)
(FOR RESEARCH ONLY)

Product: qACE™ Fast Probe qRT-PCR 4X Supermix enables robust and reliable qPCR detection of RNA and/or DNA targets, delivering superior amplification efficiency in single or multiplex assays, even from low copy target numbers in highly diluted samples. Using gene-specific primers, qACE™ Fast Probe qRT-PCR 4X Supermix allows for first-strand cDNA synthesis and subsequent qPCR in a single-tube reaction procedure, decreasing contamination risk and reducing hands-on time considerably.

The 4X concentrated Supermix comprises all components, except primers and probe, for the amplification, detection and quantification of both RNA and DNA targets in qPCR reactions based on a wide range of probe-based technologies, including TaqMan, Molecular Beacons and Scorpion probes. It consists of the combination a genetically modified thermostable MMLV reverse transcriptase with improved synthesis efficiency, lacking any RNase H activity, an advanced RNase inhibitor to impede RNA degradation, and a highly efficient hot start DNA polymerase with a novel low inhibitory technology, which prevents the formation of unwanted primer-dimers and non-specific products, thus allowing for extremely high sensitivity and specificity. qACE™ Fast Probe qRT-PCR 4X Supermix is supplied with a separate vial of ROX reference dye, so it can be used with most real-time PCR instruments.

qACE™ Fast Probe qRT-PCR 4X Supermix can be used to quantify virtually any RNA target, whether using mRNA, viral RNA or total RNA as template, including extremely low-copy number targets, with minimal effort and optimization. By omitting the cDNA synthesis step, the Supermix can also be used for the for the quantification of virtually any DNA target, including extremely low-copy number targets. And by using exon-spanning primers (for RNA) mixed with intronic primers (for DNA), one can amplify RNA and DNA targets simultaneously in a multiplex RT-qPCR. Similarly, the supermix also allows for the simultaneous detection of DNA and RNA viruses (like Adenovirus (DNA genome) and Influenza (RNA genome) in a multiplex RT-qPCR reaction.

Applications:
Genotyping
Allelic discrimination
Gene Expression Studies
Multiplex qRT-PCR
Low-copy number target gene detection
Development of *in vitro* diagnostic kits

Samples: Total RNA: 1pg-1µg; mRNA >0.01pg; viral particles: 10^1-10^8 copies; gDNA <1 µg; cDNA <100ng

Contents: One ml of qACE™ Fast Probe qRT-PCR 4X Supermix is sufficient for 200 reactions of 20µl.
Each vial is supplied with a vial containing 100X ROX (50µM).

Component	GE55s	GE55.0200	GE55.5200
qACE™ Fast Probe qRT-PCR 4X Supermix	100µl	1ml	5x 1ml
100X ROX (50µM) reference dye	50µl	200µl	5x 200µl

Properties:

Reliable and consistent quantification of low-abundance targets in single and multiplex qRT-PCR
4x concentrated mix, ideal for highly multiplexed assays
High efficiency in complex templates
Simultaneous amplification and quantification of RNA and DNA targets
Superior resistance to PCR inhibitors
Reduced primer-dimer formation
Early Ct values – Rapid extension rate
Extreme sensitivity – increased limit of detection
Allows for standard and fast cycling
Compatible with TaqMan, Scorpions and Molecular Beacons

Storage:

Store at -20°C and protected from light for at least 2 years.
Repeated thaw/freeze cycles (up to 25 times) have no negative impact on performance.

About ROX:

The reference dye ROX compensates for variations in fluorescence detection that are unrelated to the PCR reaction. The fluorescence level of ROX provides a stable baseline during cycling against which PCR-related fluorescence signals are normalized. Thus, difference between samples due to variations in reaction volumes caused by pipetting are adjusted. As the dye does not inhibit the PCR reaction and has a completely different emission spectrum, it does not interfere with qPCR on any equipment. qACE™ Fast Probe can be used with equipment requiring no ROX, low ROX or high ROX.

Prior to use:

Depending on your equipment, prior to use for the first time, add 4µl ("LowROX") or 40µl ("HighROX") [0.4 µl or 4.0 µl in case of #GE55s (trial size)] of the 100X ROX reference dye to each tube of 1ml of 4X mastermix and vortex briefly. If your instrument is "No ROX", then you should use the mastermix as is, thus without addition of ROX. Once ROX has been added, the mastermix can be used directly or stored at -20°C for up to 1 year. If you are not sure whether your instrument is "No ROX", "LowROX" or "HighROX", you can find a list at our website. If the equipment is not listed, please feel free to contact us. When handling qACE™ Fast Probe qRT-PCR 4X Supermix, minimize exposure to direct light, as exposure for an extended period of time might result in loss of signal intensity. Always certify that the product has been fully thawed and mixed well before use.

Basic Protocol:

1. Mix for each qPCR reaction:

Component	Volume	Final Conc.
qACE™ Fast Probe 4X Mastermix with ROX*)	5 µl	1X
Forward primer (10 pmol/µl)**)	0.8 -2 µl	400nM - 1µM
Reverse primer (10 pmol/µl)**)	0.8 -2 µl	400nM - 1µM
Probe (10µM)	0.25-1 µl	125-500 nM
Template RNA and/or DNA***)	1-10 µl	***)
PCR –grade water	up to 20 µl	

*) ROX as required; see section: "Prior to Use".
**) In case of multiplex, the recommended concentration of each primer should not exceed 400nM
***) Template RNA: in case of total RNA 1pg-1µg, in case of mRNA > 0.01pg, in case of viral RNA: 10^1-10^8 copies
***) Template DNA: in case of gDNA < 1µg, in case of cDNA < 100ng

In order to minimize risk of contamination, reagent loss and improve pipetting accuracy, we recommend to prepare a mastermix for multiple samples (N), always including a negative control for the detection of possible contaminants, by mixing all components (N+1), except template DNA, dividing the mixture equally into each tube and then add template DNA or PCR grade water in case of the control to the individual PCR tubes or wells of a PCR plate.

2. Set-up qRT-PCR cycling (if applicable, select fast mode on the instrument):

Nº cycles	Temp	Time	Step
1x (optional)	52ºC	5-10min for singleplex 10-20min for multiplex	cDNA synthesis (only required for RNA samples)
1x	95ºC	3min	Hot-start & RTase inactivation
40-50x	95ºC	5-15 sec	Denaturation
	55-65ºC**	20-30sec*	Annealing/Extension
Dissociation / Melt Analysis	according to manufacturer´s guidelines		

After an initial cycle of 5-20 minutes (depending on the complexity of the template and the number of targets) at 52ºC (in which cDNA is being synthesized), followed by a RTase inactivation and Polymerase activation step: cycle 40-50 times for 5-15 seconds at 95ºC, and 20-30 seconds at 55ºC-65ºC for annealing/extension. Acquire data on the appropriate channel.
*) Select the shortest time possible but not less than 20 sec and do not exceed 30 seconds
**) In case of 3-step cycling, anneal at optimal annealing temperature for 20 sec and minimum time necessary at 72ºC for data acquisition (according to manufacturer´s guidelines).

Optimization

Template

For efficient qRT-PCR, under fast cycling conditions, it is recommended to amplify cDNA fragments ranging from 80-200bp. The shorter the amplicon, the faster the reaction can be cycled. Amplicons should not exceed 400bp. High concentrations of template may inhibit PCR, result in non-specific primer binding, increase background fluorescence, and/or reduce linearity of standard curves. Results may be improved by using less template, and it is recommended to try a serial dilution to find the best concentration. It should be taken into consideration that the key factor is target copy number and not the total amount of DNA. E.g.: 1µg of human genomic DNA might contain some 200.000 copies, whereas the same amount of bacterial DNA might contain 200 million copies. For small molecules, such as cDNA, 1pg should result in a Ct around 20, whereas in order to obtain Ct of around 20 for human genomic DNA some 50 ng would be required.

Primers

Primer design and purification is of the utmost importance, especially in case of low-copy number target detection, as to minimize non-specific amplification with resulting loss of sensitivity. Primers should have melting temperatures of approximately 60ºC. To optimize results, use the lowest primer concentration that does not compromise the reaction efficiency.