qACE™ Fast Probe 4X Mastermix

#GE33.0200 (1 ml) | GE33.5200 (5x 1ml) | GE33.2502 (25x 1ml) | GE33s (trial size)
(FOR RESEARCH ONLY)

Product: qACE™ Fast Probe 4X mastermix contains all components, except primers, probe and template, for the amplification and detection and quantification of DNA in qPCR based on a wide range of probe-based technologies, including TaqMan, Molecular Beacons and Scorpion probes. It consists of the combination of an antibody-mediated highly efficient hot start enzyme with a novel low inhibitory technology, which prevents the formation of primer-dimers, thus allowing for extremely high sensitivity and specificity. qACE™ Fast Probe can be used to quantify virtually any DNA target, including extremely low-copy number targets, with minimal effort and optimization. qACE™ Fast Probe is a 4X mastermix, which is ideal for multiplex qPCR, and is supplied with a separate vial of ROX reference dye, so it can be used with most real-time PCR instruments.

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.

Applications:
Genotyping
Allelic discrimination
Multiplex qPCR
Low-copy number target gene detection
Development of *in vitro* diagnostic kits

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

Component	GE33s	GE33.0200	GE33.5200	GE33.2502
qACE™ Fast Probe 4X Mastermix	100µl	1ml	5x 1ml	25x 1ml
100X ROX (50µM) reference dye	50µl	200µl	5x 200µl	25x 200µl

Properties:
Reliable and consistent quantification of low-abundance targets in single and multiplex qPCR
4x concentrated mix, ideal for highly multiplexed assays
High efficiency in complex templates
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 -20ºC and protected from light for at least 2 years.
Repeated thaw/freeze cycles (up to 25 times) have no negative impact on performance.

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 #GE33s (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 4X Mastermix, 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 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
***) ≤1µg genomic DNA or ≤100 ng cDNA (see optimization).

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 qPCR cycling (if applicable, select fast mode on the instrument):

N° cycles	Temp	Time
1x	95°C	3min
40-50x	95°C	5-15 sec
	55-65°C**	20-30sec*
Dissociation / Melt Analysis	according to manufacturer´s guidelines	

After an initial cycle of 3 min at 95°C (Enzyme activation and denaturation of template DNA (including removal of all secondary DNA structures such as hairpins): cycle 40-50 times for 5-15 seconds at 95°C, and 20-30 seconds at 55°C-65°C 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 72°C for data acquisition (according to manufacturer´s guidelines).

Optimization

Template

For efficient qPCR, under fast cycling conditions, it is recommended to amplify DNA 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 60°C. To optimize results, use the lowest primer concentration that does not compromise the reaction efficiency.