Xpert One-Step Fast SYBR

#GE40.0100 (100 rxns) | GE40.5100 (5x 100 rxns) | GE40s (trial size)
(FOR RESEARCH ONLY)

Product: Using gene-specific primers (GSP), Xpert One-Step Fast SYBR allows for first-strand cDNA synthesis and subsequent qPCR in a single-tube reaction procedure, decreasing contamination risk and reducing hands-on time considerably. Xpert One-Step Fast SYBR consists of a RTase mix, a Fast qPCR Mastermix (SYBR), and separated vial(s) of ROX.

The RTase mix comprises a genetically modified thermostable MMLV reverse transcriptase with improved synthesis efficiency, lacking any RNase H activity, and an advanced RNase inhibitor to impede RNA degradation. The fast qPCR Mastermix (SYBR) consists of the combination of a highly efficient enzyme with a novel low inhibitory technology. The intercalating dye uses in this mastermix causes little to no inhibition of the PCR reaction thus allowing for extremely high sensitivity and specificity, as well as preventing the formation of unwanted primer-dimers and non-specific products. Xpert One-Step Fast SYBR 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.

Applications:
One-Step qPCR
Absolute quantification
Gene expression analysis
Low-copy number target gene detection

Contents: Xpert One-Step Fast SYBR is supplied with a separate vial of ROX reference dye, so it can be used with most*) real-time PCR instruments. One ml is of Mastermix is sufficient for 100 rxns of 20µl.

Component	GE40s	GE40.0100	GE40.5100
RTase Mix	25µl	100µl	5x 100µl
Fast qPCR Mastermix (SYBR)	200µl	1ml	5x 1ml
100X ROX (50µM) reference dye	50µl	0.2ml	5x 0.2ml

*) Xpert One-Step Fast SYBR can be used with equipment requiring "no ROX", "Low ROX" or "High ROX". For correct usage of ROX, see section "prior to use" on page 2. 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, differences 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. If you are not sure whether your instrument is "No ROX", "Low ROX" or "High ROX", you can find a list at our website. If the equipment is not listed, please feel free to contact us.

Properties:
Excellent signal with low PCR inhibition
Early Ct values – Rapid extension rate
Allows for standard and fast cycling

Samples: Total RNA: 10pg-100ng; mRNA >0.01pg

Storage: Store at -20°C and protected from light for at least 1 year. 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 18µl ("High ROX") [or 3.6µl in case of GE40s] of the 100X ROX reference dye to the 1-ml Fast qPCR Mastermix (SYBR) and vortex briefly. If your instrument is "Low ROX" or "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. When handling, 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.

For accurate quantification, run a standard curve, using well defined template RNA, to determine the limit of detection and the correlation between concentration and Ct, as these depend on the target. At lower concentrations, the amplification curves may begin to group together and consequently Ct values will no longer fit the standard curve. In order to be able to determine target concentration in a given sample, Ct values must fit in the linear range. If not the case, one could try to use more starting material (up to 5µg), taking into account that at very high concentrations, complete reverse transcription may be compromised.

Usage

1. **Mix for each RT-qPCR reaction**:

Component	Volume	Final Conc.
Fast qPCR Mastermix (SYBR) with ROX[1)]	10 µl	1X
Forward primer (10µM)	0.8 µl	400nM
Reverse primer (10µM)	0.8 µl	400nM
RTase Mix	1.0 µl	1X
Template RNA	see note 2	Variable
PCR –grade water (RNase-free)	up to 20 µl	

[1] ROX as required, see section: "Prior to Use".
[2] Template RNA: in case of total RNA 10pg-100ng, in case of mRNA > 0.01pg
[3] The mixtures have already been optimized. It is not recommended to add further components.

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 (no RTase) for the detection of possible contaminants, by mixing all components (N+1), except template RNA, dividing the mixture equally into each tube and then add template RNA or PCR grade water in case of the control to the individual PCR tubes or wells of a PCR plate.

2. **Set-up RT-qPCR cycling** (if applicable, select fast mode on the instrument) and acquire data on the SYBR® Green or FAM channel.

N° cycles	Temp	Time
1x (cDNA synthesis)	45°C/55°C [1)]	10 min
1x (Hot-start)	95°C	2 min
40x (qPCR)	95°C	5 sec
	60-65°C[2)]	20-30 sec[3)]
Dissociation / Melt Analysis	according to manufacturer´s guidelines	

1. For most applications, cDNA synthesis should be carried out at 45°C. In case of targets with high secondary structure, synthesis may be optimized by carrying out at 55°C
2. Cycle 40 times for 5 sec at 95°C and 20-30 sec at 60°C-65°C for annealing/extension. Do not use primers with Tm below 60°C
3. Select the shortest time possible but not less than 20 seconds and do not exceed 30 seconds. In case of 3-step cycling, anneal at optimal annealing temperature for 20 seconds and minimum time necessary at 72°C

Optimization

Template
For efficient qPCR, under fast cycling conditions, it is recommended to amplify targets ranging from 80-200bp. The shorter the amplicon, the faster the reaction can be cycled. Amplicons should not exceed 400bp. Both low and high concentrations of template may reduce linearity of standard curves. Results may be improved by using more or 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 RNA.

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 (100nM-400nM).