Xpert Fast SYBR (uni)

#GE20.0100 (100 rxns) | GE20.5100 (5x 100 rxns) | GE20.2501 (25x 100 rxns) | GE20s (trial size)

(FOR RESEARCH ONLY)

Product: Xpert Fast SYBR (uni) 2X mastermix contains all components, except primers and template, for the amplification and detection of DNA in qPCR. It consists of the combination of a highly efficient enzyme with a novel low inhibitory technology. The intercalating dye used 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 Fast SYBR (uni) can be used to quantify virtually any DNA target, including extremely low-copy number targets, with minimal effort and optimization. Xpert Fast SYBR (uni) 2X mastermix is supplied with a separate vial of ROX reference dye, so it can be used with most* real-time PCR instruments.

**Xpert Fast SYBR (uni) can be used with equipment requiring no ROX, low ROX or high ROX. If your instrument requires fluorescein, Xpert Fast SYBR (fluorescein) [#GE21.0100] should be used.*

Applications:
Absolute quantification
Gene expression analysis
High throughput PCR
Low-copy number target gene detection

Contents: One ml of Xpert Fast SYBR 2X Mastermix (uni), containing an optimized ratio of a fast hot-start DNA polymerase, dNTPs, MgCl2, and non-PCR inhibiting intercalating green dye, is sufficient for 100 rxns of 20µl. Each vial is supplied with a vial containing 100X ROX (50µM).

Component	GE20s	GE20.0100	GE20.5100	GE20.2501
Fast Fast SYBR 2X Mastermix (uni)	200µl	1ml	5x 1ml	25x 1ml
100X ROX (50µM) reference dye	50µl	150µl	5x 150µl	25x 150µl

Properties:
Excellent signal with low PCR inhibition
Early Ct values – Rapid extension rate
Extreme sensitivity – increased limit of detection
Allows for standard and fast cycling

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.

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.

Prior to use:

Depending on your equipment, prior to use for the first time, add 2µl ("LowROX") or 20µl ("HighROX") [0.4 µl or 4.0 µl in case of #GE20s (trial size)] of the 100X ROX reference dye to each tube of 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 Xpert Fast SYBR (uni), 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.
Xpert Fast SYBR 2X Mastermix (uni) with ROX*)	10 µl	1X
Forward primer (5 pmol/µl)	<2 µl	50-400nM
Reverse primer (5 pmol/µl)	<2 µl	50-400nM
Template DNA**)	<6 µl	**)
PCR –grade water	up to 20 µl	

*) ROX as required; see section: "Prior to Use". **) <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):

Nº cycles	Temp	Time
1x	95ºC	2-3min
40x	95ºC	5 sec
	60-65ºC**	20-30sec*
Dissociation / Melt Analysis	according to manufacturer´s guidelines	

After an initial cycle of 2-3 min at 95ºC (Enzyme activation and denaturation of template DNA (including removal of all secondary DNA structures such as hairpins): choose 2 min for cDNA and 3 min for complex targets), cycle 40 times for 5 seconds at 95ºC, and 20-30 seconds at 60ºC-65ºC for annealing/extension. Acquire data on the SYBR® Green or FAM 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 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. If copy numbers are really low (<100), primers are more likely to form primer dimers.

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 (50-400nM).

MgCl2

The Mg^{2+}-concentration of the Xpert Fast SYBR (uni) 2X Mastermix has already been optimized, and it is not likely that the reaction efficiency nor specificity will be improved by adding additional MgCl2.