

## Xpert Fast SYBR (Fluorescein)

#GE21.0100 (100 rxns) | GE21.5100 (5x 100 rxns) | GE21.2501 (25x 100 rxns) | GE21s (trial size)  
 (FOR RESEARCH ONLY)



**Product:** Xpert Fast SYBR (Fluorescein) 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 (Fluorescein) can be used to quantify virtually any DNA target, including extremely low-copy number targets, with minimal effort and optimization. Xpert Fast SYBR (Fluorescein) 2X mastermix contains 20nM Fluorescein and is intended for real-time PCR instruments that have a well-factor correcting feature\*

*\*) If your instrument requires ROX instead of Fluorescein as a reference dye, Xpert Fast SYBR (uni) [#GE20.0100] 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 (Fluorescein), containing an optimized ratio of a fast hot-start DNA polymerase, dNTPs, MgCl<sub>2</sub>, non-PCR inhibiting intercalating green dye, and fluorescein reference dye is sufficient for 100 rxns of 20µl.

Component	GE21s	GE21.0100	GE21.5100	GE21.2501
Fast Fast SYBR 2X Mastermix (Fluorescein)	200µl	1ml	5x 1ml	25x 1ml

**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.

### Prior to use:

If you are not sure whether your instrument requires Fluorescein (or ROX), 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 (Fluorescein), 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 (Fluorescein)	10 $\mu$ l	1X
Forward primer (5 pmol/ $\mu$ l)	<2 $\mu$ l	50-400nM
Reverse primer (5 pmol/ $\mu$ l)	<2 $\mu$ l	50-400nM
Template DNA <sup>†</sup>	<6 $\mu$ l	*)
PCR-grade water	up to 20 $\mu$ l	

\*) <1  $\mu$ g 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	2-3min
40x	95°C	5 sec
	60-65°C**	20-30sec*
Dissociation / Melt Analysis	according to manufacturer's guidelines	

After an initial cycle of 2-3 min at 95°C (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 95°C, and 20-30 seconds at 60°C-65°C 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 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.: 1  $\mu$ g 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 60°C. To optimize results, use the lowest primer concentration that does not compromise the reaction efficiency (50-400nM).

### MgCl<sub>2</sub>

The Mg<sup>2+</sup>-concentration of the Xpert Fast SYBR (Fluorescein) 2X Mastermix has already been optimized, and it is not likely that the reaction efficiency nor specificity will be improved by adding additional MgCl<sub>2</sub>.