Xpert Fast HRM

#GE70.0100 (1 ml) | GE70.5100 (5x 1ml)| GE70.2501 (25x 1ml) | GE70s (trial size)
(FOR RESEARCH ONLY)

Product: High Resolution Melt (HRM) analysis is a powerful tool for the detection of mutations and polymorphisms in dsDNA samples, including GpG methylation differences. At increasing temperature, in the presence of a fluorescent dye, tiny sequence variation between samples results in different DNA strand dissociation behaviour. HRM analysis exploits these differences using the melt curve shapes to distinguish between these samples.

Xpert Fast HRM (2X mastermix) contains all components, except specific primers and template, for HRM analysis in qPCR. The mix consists of the combination of a highly efficient enzyme with an optimized buffer system, which allows for efficient amplification of GC-rich and AT-rich templates under both normal and fast qPCR conditions. The intercalating dye used in this mastermix causes 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.

Different SNPs show different Tm melt curve shifts. Class I SNPs (C/T and G/A) demonstrate typically large shifts (>0.5ºC), whereas Class IV SNP (A/T) typically have very small shifts (<0.2ºC). The high sensitivity and specificity of Xpert Fast HRM allows to distinguish all possible SNPs.

Applications: High Resolution Melt (HRM) Analysis
SNP Genotyping
CpG methylation analysis (by comparing bisulfite-treated with control DNA)

Contents:

Component	GE70.0100	GE70.5100	GE70.2501	GE70s
Xpert Fast HRM (2X Mastermix)	1 ml	5x 1 ml	25x 1 ml	0.2ml

One ml of Xpert Fast HRM (2X mastermix), containing an optimized ratio of a fast DNA polymerase, dNTPs, MgCl2, and non-PCR inhibiting intercalating green dye, is sufficient for 100 rxns of 20µl

Properties: Excellent signal with low PCR inhibition
Ultra-sensitive melt profiles
Allows for standard and fast cycling

Storage: -20ºC and protected from light for at least 1 year (or at +4ºC for up to 1 month).

Prior to use

When handling Xpert Fast HRM, 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.

Usage

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

Component	Volume	Final Conc.
Xpert Fast HRM (2X Mastermix)	10 µl	1X
Forward primer (10 pmol/µl) [10µM]	0.8 µl	400nM
Reverse primer (10 pmol/µl) [10µM]	0.8 µl	400nM
Template DNA*)	variable	*)
PCR –grade water	up to 20 µl	

*) < 0.5-50 ng genomic DNA (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*
HRM Analysis	according to manufacturer´s guidelines	

After an initial cycle of 2-3 min at 95°C (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., 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 60°C. To optimize results, one might consider using a lower primer concentration that does not compromise the reaction efficiency (50-400nM).

$MgCl_2$

The Mg^{2+}-concentration of the Xpert Fast HRM 2X Mastermix has already been optimized (final concentration is 6mM).