GRISP RESEARCH SOLUTIONS

Xpert Hotstart Mastermix (2x) with dye

#GE28.0100 (1.25ml) | GE28.5100 (6.25ml)

(FOR RESEARCH ONLY)

Product: Xpert Hotstart DNA polymerase is a chemically modified hot-start Taq DNA polymerase, with excellent amplification efficiency, enabling higher specificity, increased sensitivity, and greater yield, as compared to standard Taq DNA polymerases, making this the ideal choice for consistent results in complex PCR amplifications and multiplex PCR.

Applications: Complex targets (including GC-rich and AT-rich templates), Multiplex PCR.

Contents: Xpert Hotstart DNA Polymerase is supplied as a convenient 2X Mastermix, containing all required components for PCR, except specific primers. It also includes an inert blue dye for electrophoresis. Final concentration of $MgCl_2$ will be 1.5mM. Each vial is supplied with an additional separated vial of $MgCl_2$ for further optimization.

Component	GE28.0100	GE28.5100
Xpert Hotstart Mastermix (2X)	1.25ml	5x 1.25ml
$MgCl_2$ (25mM)	1.5 ml	5x 1.5ml

QC: Functionally tested in PCR. Absence of endonucleases, exonucleases, and ribonucleases was confirmed by appropriate assays

Properties:

Amplicon size: up to 5kb
Extension rate: 2kb/min
Hotstart: Yes
A-overhang: Yes

Storage: -20ºC and protected from light for at least 1 year. No loss of performance is detected after 20 freeze/thaw cycles.

Prior to use:

Optimal PCR cycling conditions (incubation times and temperatures) depend on DNA target (GC-content, size, quantity, purity, etc.) and specific primers and need to be determined case by case. Xpert Hotstart Mastermix (2X) with dye includes dNTPs, $MgCl_2$ and other components to maximize success rates. We suggest to start with the following basic protocol and subsequently, if desired, optimize annealing temperature, Mg^{2+} concentration, incubation times and cycling number.

Basic Protocol

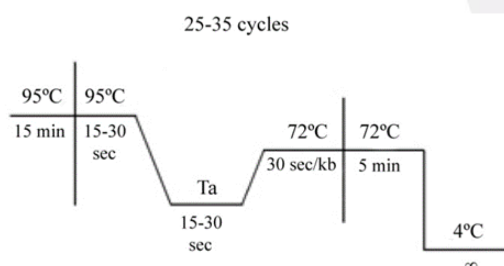
1. Mix for each PCR reaction, starting with the greatest volume (usually water) and ending with Xpert Hotstart:

Component	Volume (25µl)	Final Conc.
Xpert Hotstart Mastermix (2x) with dye	12.5 µl	1X
$MgCl_2$ (already included in PCR Buffer)	0 µl	1.5 mM
dNTPs (already included in PCR Buffer)	0 µl	0.4 mM (each)
Forward primer (5 pmol/µl)	2 µl	0.4 µM
Reverse primer (5 pmol/µl)	2 µl	0.4 µM
Template DNA*	0.25 – 10 µl	1-250 ng*
PCR–grade water	up to 25 µl	

**) In case of cDNA <50ng and in case of gDNA <250ng (total amount).*

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.

2. Set-up initial PCR amplification as follows:



After an initial cycle of 15 min at 95ºC (cleavage of the chemical moiety), cycle 25-35 times for 15-30seconds at 95ºC, 15-30 seconds at Ta, and 30 seconds per kb of target DNA at 72ºC for extension. Set the annealing temperature (Ta) as the melting temperature (Tm) of the primer with the lowest Tm. Include a final extension step of 5 min at 72ºC to ensure that all amplicons are fully extended and include 3´-A-overhang. Analyze PCR products by DNA Agarose gel electrophoresis. Samples can be loaded directly onto an agarose gel without the need of a loading buffer. On a 1% agarose gel, the blue tracking dye co-migrates with DNA of approximately 400-500bp

Optimization

$MgCl_2$
Xpert Hotstart Mastermix (2X) with dye contains 3mM MgCl2 (final concentration is 1.5mM). This product is supplied with a separated vial of 25mM MgCl2 allowing for the optimization of the final MgCl2 concentration, which is normally between 1.5mM and 3mM

Annealing Temperature (Ta) and Primers
Optimizing the annealing temperature is crucial, especially in case of multiplex PCR, as a too low temperature might result in non-specific amplification whereas a too high temperature results in no amplification. The melting temperature (Tm) is defined as the temperature in which 50% of the primer and its complementary sequence of the target DNA are present as duplex DNA. By increasing the temperature above the melting temperature, this percentage decreases, however, primers will still anneal (up to a certain point) and initiate extension. PCR can therefore be performed at temperatures of several degrees higher than Tm and it is therefore recommended to optimize the Ta by performing a temperature gradient (e.g., starting at the lowest Tm or a few degrees below and increasing with 2ºC increments). Ideally, primers have melting temperatures of approximately 60ºC and final concentration should be between 0.2 and 0.6µM (each).

Incubation times and number of cycles.
Denaturation and annealing steps may require less time depending on the thermocycler apparatus (ramp rate), reaction volume and PCR tube (varies with the efficiency of heat-transfer). It might be worthwhile to optimize (reduce) times to as low as 10 seconds for both denaturation and annealing steps, which will greatly reduce overall PCR time. Faster cycling conditions should not be applied in multiplex PCR.