Xpert Hotstart DNA Polymerase

#GE48.0500 (500 U) | GE48.2500 (2500 U) | GE48s (trial size)
(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 at a concentration of 5U/µl. For flexibility, the enzyme is supplied with a 10x reaction buffer (containing 15 mM Mg^{2+}) and an additional separated vial of $MgCl_2$.

Component	GE48.0500	GE48.2500	GE48s
Xpert Hotstart DNA polymerase (5U/µl)	100µl	5x 100µl	10µl
Xpert Hotstart Reaction Buffer (10X)	1.5 ml	5x 1.5ml	1.5 ml
$MgCl_2$ (25mM)	1.5 ml	5x 1.5ml	1.5 ml
dNTPs (10mM each)	not included	not included	not included

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, temperatures, and concentrations) depend on DNA target (GC-content, size, quantity, purity, etc.), specific primers, buffer composition, $MgCl_2$-concentration, and Xpert Hotstart-concentration and need to be determined case by case. We suggest to start with the following basic protocol and subsequently optimize annealing temperature, $MgCl_2$ concentration (note that the 10X reaction buffer already contains 15mM $MgCl_2$ in order to obtain final concentration of 1.5mM as performance at lower concentrations of Xpert Hotstart at lower concentrations is worse), incubation times, and cycling number.

Basic Protocol

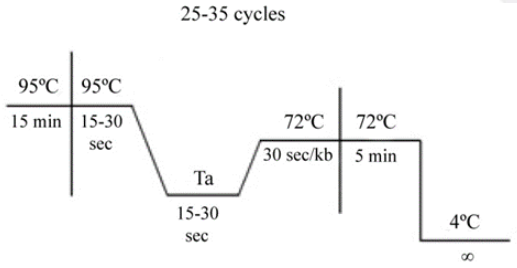
1. Mix for each PCR reaction, starting with the greatest volume (usually water) and ending with Xpert Hotstart:

Component	Volume (25µl)	Final Conc.
Xpert Hotstart Reaction Buffer (10x)	2.5 µl	1X
$MgCl_2$ (already included in PCR Buffer)	0 µl	1.5 mM
dNTPs (10mM each) [Not included in this kit]	1 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*
Xpert Hotstart DNA Polymerase (5U/µl)	0.1 µl**	0.5 U**
PCR–grade water	up to 25 µl	

**) In case of cDNA <50ng and in case of gDNA <250ng (total amount).*
***) For difficult targets, e.g., GC-rich, higher enzyme concentration may be required (up to 2.5U)*
For smaller/larger reaction volumes, scale it down/up proportionally.

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 95°C (cleavage of the chemical moiety), cycle 25-35 times for 15-30seconds at 95°C, 15-30 seconds at Ta, and 30 seconds per kb of target DNA at 72°C 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 72°C to ensure that all amplicons are fully extended and include 3´-A-overhang. Analyze PCR products by DNA Agarose gel electrophoresis.

Optimization

$MgCl_2$
Xpert Hotstart DNA Polymerase is supplied with a PCR Buffer (10x) containing 15mM MgCl2 and a separated vial of 25mM MgCl2. Taq requires MgCl2, however, the final concentration may have great influence on both quantity and specificity of the amplification and we highly recommend to optimize the final MgCl2-concentration (normally between 1.5 and 3mM in steps of 0.5mM).

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 2°C increments). Ideally, primers have melting temperatures of approximately 60°C 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.