Xpert HighFidelity

(dNTPs included)

#GE07.0250 (250 U) | GE07s (trial size)

(FOR RESEARCH ONLY)

Product: Xpert HighFidelity DNA polymerase is a robust enzyme with enhanced DNA binding, resulting in improved processivity, yield, and extremely low error-rate, ideal for applications such as high-fidelity PCR, site-directed mutagenesis, crude sample PCR, blunt-end cloning, among others, where robustness and proof-reading are important. The Xpert HighFidelity DNA polymerase has an error-rate of approximately 1 error per 4.5x107 nucleotides incorporated, which is 50x lower than Taq DNA polymerase. The velocity of Xpert HighFidelity DNA polymerase allows the use of an extension rate of up to 2kb/min, making this also an ideal choice for consistent results in fast PCR amplifications

Applications: High-Fidelity PCR, blunt-end cloning, site-directed mutagenesis

Contents: Xpert HighFidelity is supplied with 3 vials of 1ml of PCR Buffer (5x concentrated), consisting of 15mM $MgCl_2$, 5mM dNTPs, enhances and stabilizers, optimized to increased PCR success rates. #GE07 is a trial sample.

Component	GE07.0250	GE07s
Xpert HighFidelity (2U/µl)	125 µl	10 µl
PCR Buffer (5x), containing dNTPs	3x 1ml	400 µl

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

Properties:

Amplicon size: up to 10kb
Extension rate: 2kb/min
Hotstart: No
A-overhang: No
Error-rate: 50x lower than normal Taq

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, and buffer composition, $MgCl_2$-concentration, and Xpert HighFidelity-concentration and need to be determined case by case. The PCR Buffer already includes dNTPs and has been already been optimized with respect to the $MgCl_2$ concentration and other components to maximize success rates. It is not recommended to add additional $MgCl_2$ or other PCR enhancers. We suggest to start with the following basic protocol and subsequently optimize annealing temperature, incubation times and cycling number.

Basic Protocol

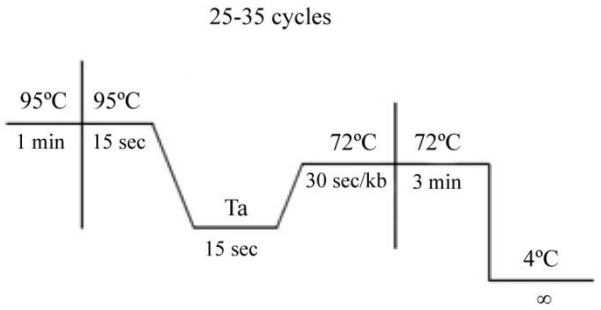
1. Mix for each PCR reaction:

Component	Volume (25µl)	Final Conc.
PCR Buffer (5x)	5 µl	1X
$MgCl_2$ (already included in PCR Buffer)	0 µl	3 mM
dNTPs (already included in PCR Buffer)	0 µl	1 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	*)
Xpert HighFidelity DNA Polymerase (2U/µl)	0.25-0.50 µl	0.02-0.04 U/µl
PCR –grade water	up to 25 µl	

*) In case of cDNA <100ng and in case of gDNA <500ng (total amount).
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 1 min at 95°C (denaturation of template DNA including removal of all secondary DNA structures such as hairpins), cycle 25-35 times for 15 seconds at 95°C, 15 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. After amplification, include a final extension step of 3 min at 72°C. Analyze PCR products by DNA Agarose gel electrophoresis.

Optimization

Annealing Temperature (Ta) and Primers
Optimizing the annealing temperature is crucial, 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 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.
Denaturation and annealing steps may require less time depending on the thermocycler apparatus, 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. For complex DNA targets it might be necessary to increase extension time from 30 seconds per kb to 60 seconds per kb.