Xpert Taq DNA Polymerase
(dNTPs included)

#GE10.0500 (500 U) | GE10.2500 (2500 U) | GE10s (trial size)
(FOR RESEARCH ONLY)

Product: Xpert Taq DNA polymerase is a robust enzyme, ideal for daily applications like genotyping and screening, amplifying with efficiency and consistency. Xpert Taq has 5'-3' exonuclease activity, but no 3'-5' exonuclease (proofreading) activity. PCR products generated with this enzyme are A-tailed, and can thus be cloned into TA cloning vectors. It is the ideal choice for consistent results in routine PCR amplifications.

Applications: Routine PCR, TA cloning.

Contents: #GE10.0500 comprises 500U and #GE10.2500 comprises 2500U of Xpert Taq DNA Polymerase, supplied at a concentration of 5U/µl. The supplied reaction buffer (5x) includes not only contain 15 mM $MgCl_2$, but also 5mM dNTPs, enhancers and stabilizers, optimized to increased PCR success rates. #GE10s is a trial sample (50U).

Component	GE10.0500	GE10.2500	GE10s
Xpert Taq (5U/µl)	100µl	5x 100µl	10µl
Xpert Taq Reaction Buffer (5X) containing dNTPs and $MgCl_2$	4x 1ml	20x 1ml	400µl

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: No
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 Taq-concentration and need to be determined case by case. Xpert Taq Reaction Buffer 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 basic protocol and subsequently optimize annealing temperature, incubation times and cycling number.

Basic Protocol

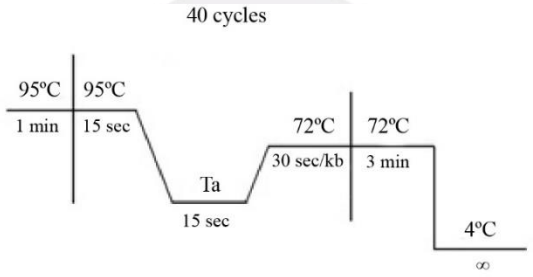
1. Mix for each PCR reaction, starting with the greatest volume (usually water) and ending with Xpert Taq:

Component	Volume (25µl)	Final Conc.
Xpert Taq Reaction 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	1-250 ng*
Xpert Taq 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 1 min at 95°C (denaturation of template DNA including removal of all secondary DNA structures such as hairpins), cycle 40 times for 15 seconds at 95°C, 15 seconds at Ta, and 30 (to 60 seconds) per kb for target DNA up to 5kb) 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 to ensure that all amplicons are fully extended and include 3´-A-overhang. 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 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. It might be worthwhile to reduce number of cycles from 40 to 25-30, depending on the success of amplification.