

Xpert Fast DNA Polymerase

#GE05.0500 (500U)
(for research only)

Product: Xpert Fast DNA polymerase is a robust enzyme, ideal for daily applications like genotyping and screening, amplifying with extreme speed, yield and consistency. Xpert Fast DNA polymerase 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. The extreme speed of Xpert Fast DNA polymerase allows the use of an extension rate of 4-8 kb/min, making this the ideal choice for consistent results in fast routine PCR amplifications.

Source: Recombinant, purified from *E.coli*

Quantity: 500U of Xpert Fast DNA Polymerase at a concentration of 5U/μl. The supplied reaction buffer (5x) includes not only 15 mM MgCl₂, but also 5mM dNTPs, enhancers and stabilizers, optimized to increased PCR success rates.

Contents:

| Product | GE05.0500 |
|---|-----------|
| Xpert Fast (5U/μl) | 100μl |
| Xpert Fast Reaction Buffer (5x), containing dNTPs and MgCl ₂ . | 4x 1ml |

Applications: Fast Routine PCR, TA Cloning

Properties: Amplicon size: up to 5kb
Extension Rate 4-8kb/min
Hotstart: No
A-overhang: Yes

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

Storage: -20°C for at least 1 year. No loss of performance is detected after 20 freeze/thaw cycles.

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Basic Protocol

Optimal PCR cycling conditions (incubation times, temperatures, and concentrations) depend on DNA target (GC-content, size, quantity, purity, etc), specific primers, buffer composition, MgCl₂-concentration, and Xpert Fast-concentration and need to be determined case by case. Xpert Fast Reaction Buffer includes dNTPs and has been already been optimized with respect to the MgCl₂ concentration and other components to maximize success rates. It is not recommended to add additional MgCl₂ or other PCR enhancers. We suggest to start with the following basic protocol and subsequently optimize annealing temperature, incubation times and cycling number.

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

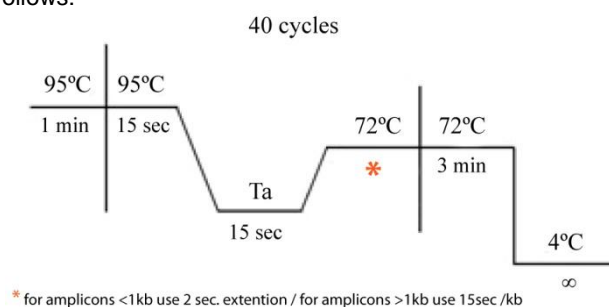
| Component | Volume (25µl) | Final Conc. |
|--|---------------|-------------|
| Xpert Fast Reaction Buffer (5x) | 5 µl | 1X |
| MgCl ₂ (already included in PCR Buffer) | 0 µl | 3 mM |
| dNTPs (already included in PCR Buffer) | 0 µl | 1 mM (each) |
| Forward primer (5 pmol/µl) | 2 µl | 0.4 µM |
| Reverse primer (5 pmol/µl) | 2 µl | 0.4 µM |
| Template DNA* | 0.25 – 10 µl | 1-250 ng* |
| Xpert Fast 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.

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 2 to 75 seconds (2 seconds for targets below 1 kb and 15 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.