# Xpert Hotstart DNA Polymerase

**#GE08.0500 (500U)**

(for research only)

**Product:** Xpert Hotstart DNA polymerase is a chemically modified hotstart 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.

**Source:** Recombinant, purified from *E.coli*

**Quantity:** 500U of Xpert Hotstart DNA Polymerase at a concentration of 5U/µl. For flexibility, the enzyme is supplied with a 10x reaction buffer (without $Mg^{2+}$) and separated vials of $MgCl_2$, and dNTPs.

**Contents:**

| Product | GE08.0500 |
|---|---|
| Xpert Hotstart DNA polymerase (5U/µl) | 100 µl |
| Xpert Hotstart Reaction Buffer (10x) | 1,5 ml |
| $MgCl_2$ (25mM) | 1,5 ml |
| dNTPs (10mM each) | 1,0 ml |

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

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

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

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

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## Basic Protocol

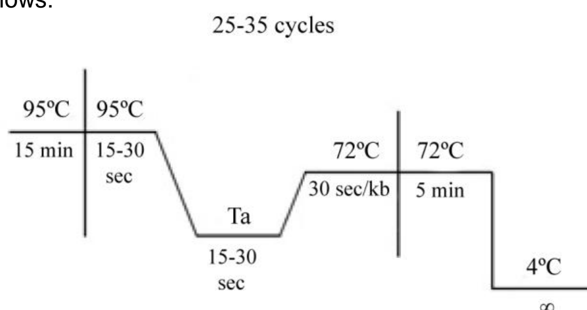
Optimal PCR cycling conditions (incubation times, temperatures, and concentrations) depend on DNA target (GC-content, size, quantity, purity, etc), specific primers, buffer composition, $MgCl_2$-concentration, and Xpert Hotstart-concentration and need to be determined case by case. We suggest to start with the following basic protocol and subsequently optimize annealing temperature, $MgCl_2$ concentration, incubation times, and cycling number.

Mix for each PCR reaction, starting with the greatest volume (usually water) and ending with Xpert Hotstart. 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. For smaller/larger reaction volumes, scale it down/up proportionally.

| Component | Volume (25µl) | Final Conc. |
|---|---|---|
| Xpert Hotstart Reaction Buffer (10x) | 2,5 µl | 1X |
| $MgCl_2$ (25mM) | 2,5 µl | 2,5 mM |
| dNTPs (10mM each) | 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 very difficult targets, e.g. higher enzyme concentration may be required (up to 2.5U)*

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.

## Optimization

**$MgCl_2$**
Xpert Hotstart DNA Polymerase is supplied with a PCR Buffer (10x) without magnesium and a separated vial of 25mM $MgCl_2$. Taq requires $MgCl_2$, however, the final concentration may have great influence on both quantity and specificity of the amplification and we highly recommend to optimize the final $MgCl_2$-concentration (normally between 1 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 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.