Xpert Taq DNA Polymerase

#GE03.0500 | # GE03.2500 | #GE13.0500 | #GE13.2500

(for research only)

Product: Xpert Taq DNA polymerase is a DNA polymerase with enhanced performance, when compared to regular Taq, and is optimized for demanding routine amplifications. The robustness of Xpert Taq DNA polymerase, as well as its high sensitivity and yield, even for targets of 8kb, makes this the ideal enzyme for daily amplifications with improved results. PCR products generated with Xpert Taq DNA polymerase contain 3´-A overhangs, and can thus be cloned into TA cloning vectors.

Source: Recombinant, purified from *E.coli*

Quantity: **#GE03.0500** and **#GE13.0500** comprises 1 vial of 500U of Xpert Taq, and **#GE03.2500** and **#GE13.2500** comprises 5 vials of 500U of Xpert Taq at a concentration of 5U/µl in 20mM Tris-HCl (pH 8.0), containing 0.1mM EDTA, 1mM DTT, 100mM NaCl, 50% glycerol, and stabilizers.

Contents: Xpert Taq is supplied with Xpert Taq Reaction Buffer (10X) [Mg^{2+}-free], Xpert Taq Reaction Buffer (10X) with dye [Mg^{2+}-free], and separated 25mM $MgCl_2$, and 100% DMSO. Using PCR Buffer with dye (bromophenol blue and xylene cyanol FF), allows for direct loading after PCR onto agarose gels, without the need of adding loading buffer. #**GE13.0500** and #**GE13.2500** are supplied with separated dNTPs.

Product	GE03.0500	GE03.2500	GE13.0500	GE13.2500
Xpert Taq (5U/µl)	100µl	5x 100µl	100µl	5x 100µl
Xpert Taq Reaction Buffer (10x)	1.5ml	5x 1.5ml	1.5ml	5x 1.5ml
Xpert Taq Reaction Buffer (10x) with dye	1.5ml	5x 1.5ml	1.5ml	5x 1.5ml
$MgCl_2$ (25mM)	1.5ml	5x 1.5ml	1.5ml	5x 1.5ml
DMSO (100%)	125µl	5x 125µl	125µl	5x 125µl
dNTPs (10mM each)	-	-	1.0ml	3x 1.0ml

Applications: Improved Routine PCR, TA Cloning, Genotyping

Properties:
Amplicon size: up to 8kb
Extension Rate 1kb/min
Hotstart: No
A-overhang: Yes

QC: Functionally tested in PCR. Bacterial DNA-free (tested by PCR). Absence of endonucleases, exonucleases, and ribonucleases was confirmed by appropriate assays.

Storage: -20ºC for at least 1 year.

GRiSP Research Solutions
Rua Alfredo Allen, 455
4200-135 Porto
Portugal
www.grisp.pt | info@grisp.pt

Basic Protocol

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 Taq-concentration, and need to be determined case by case. We suggest to start with the following basic protocol and subsequently optimize $MgCl_2$-concentration, annealing temperature, incubation times and cycling number.

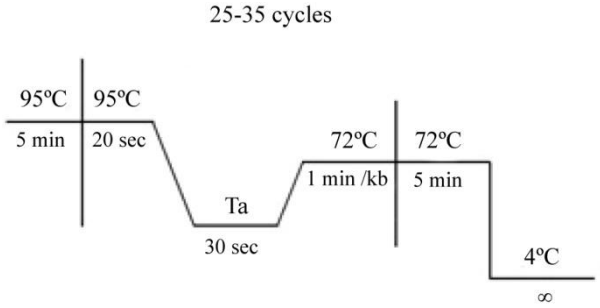
Mix for each PCR reaction, starting with the greatest volume (usually water) and ending with Xpert Taq:

Component	Volume (25µl)	Final Conc.
$MgCl_2$ (25mM)	1.5 µl	1.5 mM
dNTPs (10 mM each)	1 µl	0.4 mM
Forward primer (5 pmol/µl)	1.5 µl	0.3 µM
Reverse primer (5 pmol/µl)	1.5 µl	0.3 µM
PCR Buffer (10x) (with or without dye)	2.5 µl	1X
Template DNA	0.25 – 10 µl	2-300 ng*
Xpert Taq DNA Polymerase (5U/µl)	0.1 – 0.5 µl	0.5 -2.5U
DMSO (100%)	0 – 2.5µl	0-10%**
PCR–grade water	up to 25 µl	

**) In case of plasmid DNA use 1-10 ng, in case of gDNA add up to 100-300ng*
***) For difficult targets, e.g. GC-rich, or supercoiled plasmids, DMSO can be added as an enhancer as it disrupts DNA structures and facilitates primer annealing (note that Tm will decrease with several degrees). As it inhibits polymerase activity, it should not be used routinely.*
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 PCR amplification as follows:



After an initial cycle of 5 min at 95ºC (complete denaturation of template DNA including removal of all secondary DNA structures such as hairpins), cycle 25-35 times for 20 seconds at 95ºC, 30 seconds at Ta and 1 min (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. After amplification, 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. Note that in case of using the PCR Buffer with dyes, one can load directly onto an agarose gel, without the need of adding loading buffer.

Optimization

Annealing Temperature (Ta)
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 2ºC increments). Ideally, primers have melting temperatures of approximately 60ºC and final concentration should be between 0.2 and 0.6µM (each).

$MgCl_2$
Xpert Taq 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).

Incubation times
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.