Xpert Taq Mastermix (2X) with dye

#GE14.0100 (1,25 ml) | #GE14.5100 (5x 1,25 ml)

(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. Upon completion of PCR, the reaction is ready for direct loading onto an agarose gel without the need of adding loading buffer.

Source:

Recombinant, purified from *E.coli*

Quantity:

Xpert Taq is supplied as a convenient 2x mastermix, including blue tracking dyes (Bromophenol Blue and Xylene Cyanol) for electrophoresis, containing all required components for PCR, except specific primers. 1,25 ml is suitable for 100 reactions of 25µl.

#GE14.0100 contains 1,25 ml of Xpert Taq Mastermix (2X) with dye and
#GE14.5100 contains 5 vials of each 1,25 ml Xpert Taq Mastermix (2X) with dye

Contents:

Xpert Taq Mastermix (2X), which already contains $MgCl_2$ (final concentration will be 2mM) is supplied with additional separated 25mM $MgCl_2$ allowing further optimization, and 100% DMSO for GC-rich targets.

Product	GE14.0100	GE14.5100
Xpert Taq Mastermix(2x) with dye	1,25 ml	5x 1,25 ml
$MgCl_2$ (25mM)	100 µl	5x 100 µl
DMSO (100%)	50 µl	5x 50 µl

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.

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Basic Protocol

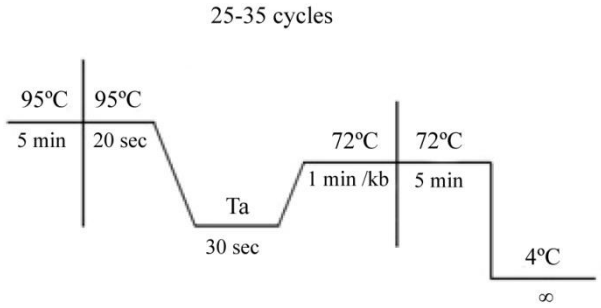
Optimal PCR cycling conditions (incubation times and temperatures) depend on DNA target (GC-content, size, quantity, purity, etc) and specific primers and need to be determined case by case. Xpert TaqMastermix (2X) includes dNTPs and $MgCl_2$ and other components. Success rates might be improved by adding additional $MgCl_2$ or other PCR enhancers (e.g. DMSO). We suggest to start with the following basic protocol and subsequently optimize annealing temperature, incubation times and cycling number.

Mix for each PCR reaction according to the following table. 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 Taq Mastermix (2X) with dye	12.5 µl	1X
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*
DMSO (100%)	0 – 2.5µl	0-10%**
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, 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

Set-up initial 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. Samples can be loaded directly onto an agarose gel without the need of adding a loading buffer.

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 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 Mastermix (2X) with dye contains 4mM $MgCl_2$ (final concentration is 2mM). Quantity and specificity of the amplification may be improved by increasing $MgCl_2$-concentration.

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.