Xpert Fast Mastermix (2X) with dye

#GE15.0001 (1 ml) | #GE15.5001 (5x 1ml)

(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. 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 Fast is supplied as a convenient 2x mastermix, including an inert red tracking dye for electrophoresis, containing all required components for fast PCR, except specific primers. Final concentration of $MgCl_2$ will be 3mM. One ml is suitable for 80 reactions of 25µl (or 100 reactions of 20µl).

#GE15.0001 contains 1 ml of Xpert Fast Mastermix (2X) with dye and
#GE15.5001 contains 5 vials of each 1 ml Xpert Fast Mastermix (2X) with dye

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

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Basic Protocol

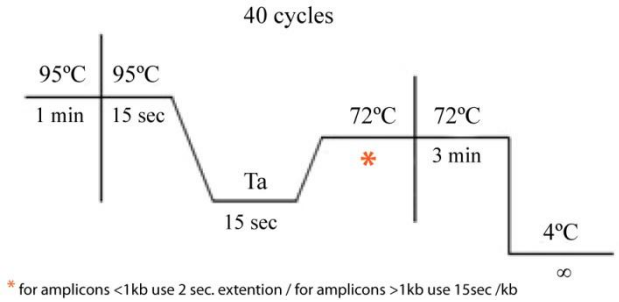
Optimal PCR cycling conditions (incubation times and temperatures) depend on DNA target (GC-content, size, quantity, purity, etc) and specific primers and need to be determined case by case. Xpert Fast Mastermix (2X) includes dNTPs and has already been optimized with respect to the $MgCl_2$ concentration 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 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 Fast 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*
PCR –grade water	up to 25 µl	

*) In case of cDNA <50ng and in case of gDNA <250ng (total amount).
For smaller/larger reaction volumes, scale it down/up proportionally.

Set-up initial PCR amplification as follows:



After an initial cycle of 1 min at 95ºC (denaturation of template DNA including removal of all secondary DNA structures such as hairpins), cycle 40 times for 15 seconds at 95ºC, 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 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 3 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. Using a 1% agarose gel, the inert red tracking dye co-migrates with DNA of approximately 600bp and using a 2% with DNA of approximately 350bp.

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).

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.