Xpert Fast Hotstart Mastermix (2X) with dye

#GE45.0001 (1 ml) | #GE45.5001 (5x 1ml)

(for research only)

Product: Xpert Fast Hotstart DNA polymerase is a robust enzyme, suitable for the amplification of difficult targets with extreme speed (4-8kb/min), yield and specificity. The buffer composition has been optimized via high-throughput screening and makes the enzyme particularly resistant to PCR inhibitors. As a result, Xpert Fast Hotstart DNA Polymerase is suitable for direct PCR from unpurified samples, including blood, urine, and bacterial colonies, making this the ideal choice for consistent results in fast complex 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 Hotstart 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).

#GE45.0001 contains 1 ml of Xpert Fast Hotstart Mastermix (2X) with dye and **#GE45.5001** contains 5x 1 ml of Xpert Fast Hotstart Mastermix (2X) with dye

Applications: Direct PCR, Fast amplification of complex targets (including GC-rich and AT-rich templates), Colony PCR, Multiplex PCR.

Properties:

Amplicon size: up to 5kb
Extension Rate 4-8kb/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

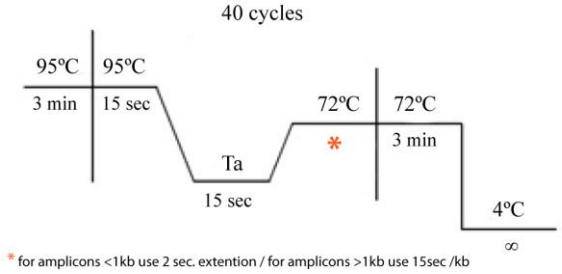
Optimal PCR cycling conditions (incubation times and temperatures) depend on DNA target (GC-content, size, quantity, purity, etc) and specific primers and need to be determined case by case. Xpert Fast Hotstart Mastermix (2x) with dye includes dNTPs and has already been optimized with respect to the $MgCl_2$ 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 Hotstart 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).*
In case of Direct PCR: use 1µl of mammalian blood or urine per 25µl reaction volume
In case of Colony PCR: use a sterile tip to resuspend a colony (or 5µl of an overnight culture) directly in the PCR reaction mix.

Set-up initial PCR amplification as follows:



After an initial cycle of 3 min at 95ºC (enzyme activation, denaturation of template DNA), cycle 40 times for 15 seconds at 95ºC, 15 seconds at Ta, and 2 to 75 seconds at 72ºC for extension (2 seconds for targets below 1 kb and 15 seconds per kb for target DNA up to 5kb | or 90 seconds total per cycle in case of multiplex PCR, independent of the sizes of the amplicons). Set the annealing temperature (Ta) as the melting temperature (Tm) of the primer with the lowest Tm. 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.
NOTE: In case of Colony PCR, increase initial denaturation time to 10 minutes.

Optimization

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. **Fast cycling conditions should not be applied in multiplex PCR.** The initial recommended 90 seconds per cycle may be further extended to increase yield. It might be worthwhile to reduce number of cycles from 40 to 25-30, depending on the success of amplification.