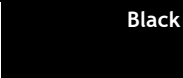


FAST HotStart DNA polymerase (2X) MasterMix with dye

FAST hotstart DNA polymerase (2X) MasterMix with dye for direct loading onto agarose gels amplifies with extreme speed, yield, and consistency.

Component	Cat#	M3286.0001	M3286.0005	Colour code of cap
FAST HotStart DNA polymerase (2X) MasterMix with dye for direct loading onto agarose gels amplifies with extreme speed, yield, and consistency.		1mL	5x 1mL	

Product description

Genaxxon's FAST HotStart DNA polymerase (2X) MasterMix with dye is the master mix of our FAST HotStart DNA polymerase which is a robust enzyme, ideally suited for applications like genotyping and screening, amplifying with extreme speed of up to 2 seconds per 1kb, as well as high yield and consistency. FAST HotStart 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 Genaxxon's FAST HotStart DNA polymerase allows the use of an extension rate of 4-8 kb/min. Genaxxon bioscience's FAST HotStart DNA Polymerase (2X) MasterMix with dye includes all components needed for a fast PCR, except the primers. Besides this, it includes an inert red tracking dye for gel electrophoresis. Therefore, upon completion of the PCR, the reaction is ready for direct loading onto an agarose gel without the need of adding an additional loading buffer.

Product Specifications

Extension rate:	Up to 30 kb/min. at 72°C
Amplicon size	Up to 5kb
Hotstart	Yes
5'-3' exonuclease activity:	Yes
Extra addition of A:	Yes
3'-5' exonuclease activity:	No
Nuclease contamination:	No
Protease contamination:	No
RNase contamination:	No

Source

Recombinant, purified from *E.coli*

Applications

Fast Routine PCR
 TA Cloning
 Colony screenings
 Genotyping

Product quantity

Genaxxon's FAST HotStart DNA polymerase (2X) MasterMix with dye is supplied as a convenient 2x master mix, including an inert red tracking dye for electrophoresis, containing all required components for fast PCR, except specific primers. Final concentration of MgCl₂ will be 3mM. One mL is suitable for 80 reactions of 25µL (or 100 reactions of 20µL).

Quality Control

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

Storage

FAST DNA polymerase (2X) MasterMix with dye should be stored immediately upon receipt at -20°C. When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance. No loss of performance is detected after 20 freeze/thaw cycles.

Product Use Limitations

FAST DNA polymerase (2X) MasterMix with dye is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@genaxxon.com).

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com)

PCR Protocol Part

Protocol using FAST HotStart DNA Polymerase (2X) MasterMix with dye

This protocol serves as a guideline for PCR amplification. Optimal cycling conditions such as incubation times, temperatures, and amount of template DNA depend on the DNA target (GC-content, size, quantity, purity, etc.), primers, buffer composition, MgCl₂-concentration and enzyme concentration and must be determined individually. We recommend starting with the basic protocol described below and subsequently optimize the annealing temperature, incubation times and cycling numbers.

Please note: The FAST HotStart DNA Polymerase (2X) MasterMix with dye already includes dNTPs and has been optimized with respect to the MgCl₂-concentration and other components to guarantee highest success rates. Therefore, we do not recommend adding additional MgCl₂ or other enhancers.

Important notes before getting started

Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis. Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Procedure

- 1. Prepare a master mix according to Table 1.**
To minimize the risk of contamination or reagent loss and to improve pipetting accuracy, we recommend preparing a master mix for multiple samples containing all the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included in every experiment.
- 2. Mix your master mix with all components except the template DNA.** Keep the master mix on ice.

Table 1: PCR reaction components (25µL PCR reaction)

Components	Quantities	Final Concentration
FAST HotStart MasterMix (2X) with dye	12.5µL	1X
Forward primer (5pmol/µL)	2µL	0.4µM
Reverse primer (5pmol/µL)	2µL	0.4µM
Template DNA	0.25 - 10µL	1 - 250ng*
PCR grade water	up to 25µL	

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

- 3. Mix the master mix thoroughly** and dispense appropriate volumes into PCR tubes. Mix gently, for example, by pipetting the master mix up and down. Do not vortex as this may harm the enzyme. It is recommended that PCR tubes are kept on ice before placing in the thermal cycler.
- 4. Add template DNA** (or PCR grade water in case of the control) to the individual tubes containing the master mix.
- 5. Program the thermal cycler** according to the manufacturer's instructions. A typical PCR cycling program is outlined in Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.
- 6. Place PCR tubes in the thermal cycler** and start program.
- 7. Analyse PCR products by agarose gel electrophoresis.** Samples can be loaded directly onto the gel without the need of adding an additional loading buffer. Using a 1% agarose gel, the inert red tracking dye co-migrates with DNA of approximately 600bp. When using a 2% agarose gel, the tracking dye co-migrates with DNA of approximately 350bp.

Table 2: PCR conditions (Thermal cycler)

Step	time	temperature	comments
Initial denaturation:	1 min	95 °C	denaturation of template DNA including removal of all secondary DNA structures such as hairpins
3-step cycling			
Denaturation:	15 sec	95 °C	
Annealing:	15 sec	Ta*	Ta* should be 2 °C below the Tm** value of the primer with the lowest Tm
Extension:	2-15 sec***	72 °C	2 sec for targets <1kb, 15sec/kb for targets >1kb
Number of Cycles	35 - 40		
Final extension	3 min	72 °C	

* Ta is the annealing temperature

** Tm is the melting temperature of a primer which is defined as the temperature at which 50% of the primer bind to the complementary sequence of the target DNA.

Please note: By increasing Ta above Tm, this percentage decreases, however, primers will still anneal up to a certain degree and initiate extension. Therefore, PCR would still work with a Ta of several degrees higher than the Tm but with a dramatically reduced efficiency. Hence, we recommend optimizing the Ta by performing a temperature gradient (e.g., starting at the lowest Tm or a few degrees below and increasing with 2 °C increments).

*** Largest tested amplicon size: 5kb. Please note that longer extension times may be required for targets larger than 5kb!

Recommendations

- **Annealing temperature (Ta)**
Optimization of the annealing temperature is important to guarantee an optimal amplification. We recommend performing a temperature gradient and using primers with a Tm >60 °C.
- **Primer concentration**
The final primer concentration should be in the range of 0.2 μM to 0.6 μM.

Optimization potential for incubation times and number of cycles

Denaturation and annealing steps may require less time depending on the thermocycler you're using (ramp rate), the reaction volume, the PCR tube (varies with the efficiency of heat transfer) and your template primer combination.

Therefore, it might be worthwhile to optimize times and cycle number depending on the success of your amplification. To greatly reduce the overall PCR time, you can try to:

- reduce times to as low as 10 seconds for both steps, annealing and denaturation.
- reduce the number of cycles to 25-30.

Trouble shooting

Non-specific amplification	
Ta too low	Increase Ta in 2 °C increments. Choose primers with a Tm above 60 °C.
MgCl ₂ concentration too high	Decrease MgCl ₂ if possible.
Primer concentration too high	Final concentration should be between 0.2 and 0.6 μM for each primer.
No amplification	
Ta too high	Decrease Ta in 2 °C increments.
MgCl ₂ concentration too low	Increase MgCl ₂ concentration.
Forgot to pipet template DNA	Make sure that template DNA and primers are included and repeat PCR.
Primer concentration too low	Final concentration should be between 0.2 and 0.6 μM for each primer.