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FAST DNA polymerase

FAST DNA polymerase is a robust enzyme amplifying with extreme speed, yield, and consistency

	Cat#	M3285.0500	M3285.2500	Colour code of cap
Component				
FAST polymerase (5U/µL)		500 units	5x 500 units	
FAST Reaction Buffer (5x), containing dNTPs and $MgCl_2$		4x 1mL	20x 1mL	

Product description

Genaxxon's FAST DNA polymerase 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 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 DNA polymerase allows the use of an extension rate of 4-8 kb/min. Genaxxon bioscience's FAST DNA Polymerase is delivered with 5X reaction buffer already containing $MgCl_2$, dNTPs, enhancers, and stabilizers for an optimal PCR result.

Product Specifications

Concentration:	5 units/µL
Extension rate:	Up to 30 kb/min. at 72°C
Amplicon size	Up to 5kb
Hotstart	No
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

500U or 2500U of FAST DNA Polymerase are supplied at a concentration of 5U/µl. The supplied reaction buffer (5x) includes not only 15mM $MgCl_2$, but also 5mM dNTPs, enhancers, and stabilizers, optimized to increase PCR success rates.

Quality Control

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

Storage

FAST DNA polymerase, including buffers and reagents, 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.

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Product Use Limitations

FAST DNA polymerase 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)

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PCR Protocol Part

Protocol using FAST DNA Polymerase

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, $MgCl_2$-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 Reaction Buffer already includes dNTPs and has been optimized with respect to the $MgCl_2$-concentration and other components to guarantee highest success rates. Therefore, we do not recommend adding additional $MgCl_2$ 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** starting with the component with the greatest volume (usually water) and ending with the FAST DNA polymerase. Keep the master mix on ice.

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

Components	Quantities	Final Concentration
FAST Reaction Buffer	5µL	1X
Forward primer (5pmol/µL)	2µL	0.4µM
Reverse primer (5pmol/µL)	2µL	0.4µM
Template DNA	0.25 – 10µL	1 – 250ng*
FAST DNA polymerase	0.1µL**	0.5U**
PCR grade water	up to 25µL	

* In case of cDNA <50ng and in case of gDNA <250ng (total amount)
** For difficult targets, e.g., GC-rich, higher enzyme concentration may be required (up to 2.5U).
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.

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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 0.2µM to 0.6µM.

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.
	Taq starts to work at ambient temperature	Use a hotstart Taq polymerase.
	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.