NIPPON Genetics EUROPE GmbH

FastGene® Taq DNA Polymerase

Technical Data Sheet

Product Description

FastGene® Taq DNA Polymerase is the single-subunit *Taq* DNA polymerase of the thermophilic bacterium *Thermus aquaticus*, purified from recombinant *Escherichia coli*.

FastGene® Taq DNA Polymerase has 5'-3' polymerase and 5'-3' exonuclease activity, but no 3'-5' exonuclease (proofreading) activity. The enzyme system has an error rate of approximately 1 error per 2.2 x 10^5 nucleotides incorporated. PCR products generated with FastGene® Taq are A-tailed and are suitable for cloning into TA cloning vectors.

FastGene® Taq DNA Polymerase is supplied with two novel reaction buffers (Buffer A and Buffer B) designed for optimal enzyme activity and performance. The proprietary formulation facilitates specific primer annealing, which translates to higher yields and specific product when compared to traditional Taq buffers.

Product Applications

FastGene® Taq DNA Polymerase is ideally suited for:

- Standard PCR applications
- DNA labelling
- DNA sequencing
- Any application requiring a high quality, thermostable DNA polymerase

Product Specifications

Shipping and Storage

FastGene® Taq kits are shipped on ice packs. Upon arrival, store kit components at -20 °C in a constant-temperature freezer. When stored under these conditions and handled correctly, full activity of the kit is retained until the expiry date indicated on the kit label.

Handling

Always ensure that the product has been fully thawed and mixed before use. Avoid freeze-thaw cycles. If these are expected, please prepare aliquots.

Quality Control

Protein purity of FastGene® Taq DNA Polymerase is confirmed by SDS-PAGE, in which more than 95% of protein must be homogenous in purfied state.

Kit Codes and Components		
LS21 **LS22** LS21NTP	**FastGene® Taq DNA Polymerase** **FastGene® Taq DNA Polymerase** FastGene® Taq DNA PCR Kit	**500 Units** **2000 Units** 500 Units + dNTPs
Related Products		
LS26 LS27 LS33 LS35	FastGene® TAQ Ready Mix PCR Kit FastGene® TAQ Ready Mix PCR Kit FastGene® BAC free HS TAQ FastGene® apTaq HotStart Polymerase	50 x 50µl rxns 250 x 50µl rxns 500 Units 500 Units
Direct PCR		
LS05 LS06 LS29	DNAreleasy Advance DNAreleasy Advance FastGene® Optima HotStart Ready Mix	10 preps 50 preps 500 x 25 rxns

Quick Notes

- FastGene® Taq DNA Polymerase can replace any commercial Taq DNA polymerase in an existing protocol. The final $MgCl_2$ concentration may need to be optimized to account for differences in buffer formulation.
- Both Buffer A and Buffer B contain $MgCl_2$ at a final concentration of 1.5 mM.
- Buffer A is recommended as first approach and for applications requiring high yields.
- Buffer B is recommended for applications where high sensitivity is required (e.g. when the template is limiting).
- Both buffers may be evaluated to determine the buffer most suitable for a specific application.
- The FastGene® Taq PCR system is suitable for the amplification of fragments up to 3.5 kb from genomic DNA or 5 kb from less complex targets.

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FastGene® Taq PCR Protocol

FastGene® Taq DNA Polymerase can be used to replace any commercial Taq DNA polymerase in an existing protocol. To allow the most seamless integration of FastGene® Taq into existing protocols, be sure to match reaction conditions, particularly the $MgCl_2$ concentration, as closely as possible.

Step 1: Prepare the PCR master mix

- Ensure that all reagents are properly thawed and mixed.
- Calculate the required volumes of each component based on the following table:

Components	50 µl reaction[1]	Final conc.
PCR-grade water	Up to 50 µl	N/A
5 U/µl FastGene® Taq DNA Polymerase	0.2 µl	1 U[2]
10X Buffer A or B[3]	5.0 µl	1X
2 mM dNTP	5.0 µl	200 µM
25 mM $MgCl_2$	As required[4]	>1.5 mM
10 µM Forward Primer	2.0 µl	0.4 µM
10 µM Reverse Primer	2.0 µl	0.4 µM
Template DNA	As required	As required[5]

[1]Reaction volumes of 10-50 µl are recommended. For volumes smaller than 50 µl scale reagents down proportionally.

[2]For GC-rich and other difficult templates, higher enzyme concentrations (up to 5 U per 50 µl reaction) may be required.

[3]Buffer A and Buffer B contain a final $MgCl_2$ concentration of 1.5 mM at 1X.

[4]For assays requiring >1.5 mM $MgCl_2$, the reaction may be supplemented with additional $MgCl_2$ as required.

[5]≤250 ng for genomic DNA; ≤25 ng for less complex DNA (e.g. plasmid, lambda).

- Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of reactions to be performed.

Step 2: Set up individual reactions

- Transfer the appropriate volume of PCR master mix, template and primer to individual PCR tubes/wells of a PCR plate.
- Cap or seal individual reactions, mix and centrifuge briefly.

Step 3: Run the PCR

- Confirm that the PCR cycling protocol conforms to the following parameters:

Step	Temperature	Duration	Cycles
Initial denaturation	95 ºC	3 min[1]	1
Denature	95 ºC	30 sec	35[3]
Anneal	T_m - 5 °C[2]	30 sec	
Extent	72 °C	1 min/kb	
Final extension	72 °C	1 min/kb	1

[1]Initial denaturation for 3 min at 95 °C is recommended for most assays. For GC-rich targets (>65% GC content) 5 min at 95 °C may be used.

[2]An annealing temperature 5 °C lower than the calculated melting temperature (T_m) of the primer set is recommended as first approach. If low yields and/or non-specific amplification is obtained, an annealing temperature gradient PCR is recommended to determine the optimal annealing temperature for the primer set empirically within the FastGene® Taq PCR system.

[3]35 cycles are sufficient for most assays. A higher number of cycles may be necessary for assays requiring a higher level of sensitivity.

For technical support please contact:
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