NIPPON Genetics EUROPE GmbH

FastGene® Taq HotStart PCR Kit

Technical Data Sheet

Product Description

FastGene® Taq DNA Polymerase is the single-subunit, wild-type Taq DNA polymerase from the thermophilic bacterium Thermus aquaticus, produced from recombinant Escherichia coli. FastGene® Taq has 5'-3' polymerase and 5'-3' exonuclease activities, but no 3'-5' exonuclease (proofreading) activity. The enzyme has an error rate of approximately 1 error per 2.2 x 10^5 nucleotides incorporated.

In the HotStart formulation, the enzyme is combined with a proprietary antibody that inactivates the enzyme until the first denaturation step. This prevents nonspecific amplification during reaction setup, increases sensitivity, and improves reaction efficiency. PCR products generated with FastGene® Taq HotStart are A-tailed and may be cloned into TA cloning vectors.

FastGene® Taq HotStart Buffer is uniquely formulated buffer to facilitate specific primer annealing. This translates to higher yields of specific product when compared to traditional Taq buffers, and improved amplification of GC- and AT-rich templates. However, FastGene® Taq HotStart DNA Polymerase may be used in combination with any standard Taq buffer with a pH of 8.3 or higher.

Product Applications

The FastGene® Taq HotStart PCR Kit is ideally suited for:

- Routine PCR
- Multiplex PCR
- Amplification of DNA for Sanger sequencing
- Any standard PCR application for which a hot start formulation of a high-quality thermostable DNA polymerase is required.

Product Specifications

Shipping and Storage

FastGene® Taq HotStart PCR 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. FastGene® Taq HotStart Buffer contains isostabilizers and may not freeze solidly, even when stored at -20 °C. This will not affect the shelf-life of the product.

Kit Codes and Components		
LS23	FastGene® HotStart TAQ DNA Polymerase	100 Units
LS24	FastGene® HotStart TAQ DNA Polymerase	250 Units
LS25	FastGene® HotStart TAQ DNA Polymerase	1000 Units
Related Products		
LS20	FastGene® Taq DNA Polymerase	100 Units
LS21	FastGene® Taq DNA Polymerase	500 Units
LS22	FastGene® Taq DNA Polymerase	2000 Units
LS26	FastGene® TAQ Ready Mix PCR Kit	50 x 50µl rxns
LS27	FastGene® TAQ Ready Mix PCR Kit	250 x 50µl rxns
Direct PCR		
LS05	DNAreleasy Advance	10 preps
LS06	DNAreleasy Advance	50 preps
LS07	FastGene® Direct PCR Kit	10 preps/20 PCR rxns
LS08	FastGene® Direct PCR Kit	50 preps/100 PCR rxns
LS09	FastGene® Direct PCR Kit	50 preps/200 PCR rxns
LS10	FastGene® Direct PCR Kit	10 preps/100 PCR rxns

Quick Notes

- FastGene® Taq HotStart DNA Polymerase can replace any commercial hot start Taq DNA polymerase in an existing protocol. The final $MgCl_2$ concentration and annealing temperature may need to be optimized to account for differences in formulation.
- The FastGene® Taq HotStart Buffer is a uniquely-formulated buffer offering improved specificity and sensitivity, and improved amplification of GC- and AT-rich templates.
- The FastGene® Taq HotStart Buffer does not contain $MgCl_2$; $MgCl_2$ (25 mM) is supplied separately to allow greater flexibility during reaction setup.
- The FastGene® Taq HotStart PCR Kit is suitable for the amplification of fragments up to 3.5 kb from genomic DNA or 5 kb from less complex targets.

Handling

Always ensure that the product has been fully thawed and mixed before use. Reagents may be stored at 4 °C for short-term use (up to 1 month). Return to -20 °C for long-term storage.

Quality Control

Each batch of FastGene® Taq HotStart DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). The FastGene® Taq HotStart PCR kits are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activity, and meet strict requirements with respect to DNA contamination levels.

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FastGene® Taq HotStart PCR Protocol

FastGene® Taq HotStart DNA Polymerase can be used to replace any commercial hot start 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 $MgCl_2$, primer and enzyme concentrations, as closely as possible.

Step 1: Prepare the PCR master mix

- Ensure that all reagents are properly thawed and mixed.
- Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of the reactions to be performed.
- Calculate the required volumes of each component based on the following table:

Component	50 µl rxn[1]	Final conc.
PCR-grade water	Up to 50 µl	N/A
5 U/µl FastGene® Taq HotStart DNA Polymerase[2]	0.2 µl	1 U
5X FastGene® Taq HotStart Buffer	10.0 µl	1X
25 mM $MgCl_2$	3.0 µl	1.5 mM[3]
dNTP Mix (10 mM each)	1.0 µl	0.2 mM each
Forward Primer (10 µM)	0.5 – 2.5 µl	0.2 – 0.5 µM
Reverse Primer (10 µM)	0.5 – 2.5 µl	0.2 – 0.5 µM
Template DNA[4]	As required	As required

[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] A final $MgCl_2$ concentration of 1.5 mM is sufficient for most standard applications. For assays that do not perform well with 1.5 mM $MgCl_2$, the optimal $MgCl_2$ concentration for each primer–template combination should be determined empirically.

[4] ≤250 ng for genomic DNA; ≤25 ng for less complex DNA (e.g. plasmid, lambda).

NOTE: For GC-rich or other difficult templates or amplicons, include DMSO at a final concentration of 5%.

Step 2: Set up individual reactions

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

Step 3: Run the PCR

- Perform PCR with the following cycling protocol:

Step	Temperature	Duration	Cycles
Initial denaturation	95 °C	3 min[1]	1
Denature	95 °C	30 sec	35[3]
Anneal[2]	T_m – 5 °C	30 sec	
Extension	72 °C	1 min/kb	
Final extension (optional)[4]	72 °C	1 min/kb	1
Store	4 – 10 °C	HOLD	1

[1] Initial denaturation for 3 min at 95 °C is recommended for most assays. For GC-rich targets (>65% GC), 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 a first approach. If low yields and/or nonspecific amplification is obtained, an annealing temperature gradient PCR is recommended to determine the optimal annealing temperature of the primer pair.

[3] 35 cycles are sufficient for most assays. A higher number of cycles may be necessary for assays requiring higher sensitivity, while lower cycle numbers can be used if the template copy number is high.

[4] Final extension should be included if PCR products are to be cloned into TA cloning vectors.

For information on product use limitations and licenses:
http://nippongenetics.eu/contact/terms/

For technical support please contact:
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