NIPPON Genetics EUROPE GmbH

FastGene® Taq ReadyMix 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. PCR products generated with FastGene® Taq are A-tailed and may be cloned into TA cloning vectors.

FastGene® Taq ReadyMix (2X) is a ready-to-use cocktail containing all components for PCR, except primers and template. The 2X ReadyMix contains FastGene® Taq DNA Polymerase (1 U per 50 µl reaction), FastGene® Taq Buffer (1X), dNTPs (0.2 mM of each dNTP at 1X), $MgCl_2$ (1.5 mM at 1X) and stabilizers.

FastGene® Taq ReadyMix is available with and without dye. The 2X ReadyMix with dye contains two inert tracking dyes to enable direct loading of PCR products onto agarose gels for analysis by electrophoresis, without the need to add a DNA loading solution.

Product Applications

FastGene® Taq ReadyMixes are ideally suited for:

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

FastGene® Taq ReadyMix with dye is recommended when reaction products are analyzed by agarose gel electrophoresis.

Kit Codes and Components		
LS26	FastGene® TAQ Ready Mix PCR Kit	50 x 50µl rxns
LS27	FastGene® TAQ Ready Mix PCR Kit	250 x 50µl rxns
Related Products		
LS23	FastGene® HotStart TAQ DNA Polymerase	100 Units
LS24	FastGene® HotStart TAQ DNA Polymerase	250 Units
LS25	FastGene® HotStart TAQ DNA Polymerase	1000 Units
LS20	FastGene® Taq DNA Polymerase	100 Units
LS21	FastGene® Taq DNA Polymerase	500 Units
LS22	FastGene® Taq DNA Polymerase	2000 Units
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 ReadyMix can replace any commercial Taq DNA polymerase in an existing protocol. The annealing temperature may need to be optimized to account for differences in formulation.
- 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.
- The 2X FastGene® Taq ReadyMix includes two inert tracking dyes, which allow loading of PCR products directly onto agarose gels for analysis.
- FastGene® Taq ReadyMixes contain 1.5 mM $MgCl_2$ and 0.2 mM of each dNTP (at 1X).

Product Specifications

Shipping and Storage

FastGene® Taq ReadyMix 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 ReadyMixes contain isostabilizers and may not freeze solidly, even when stored at -20 °C. This will not affect the shelf-life of the product.

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 DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). FastGene® Taq ReadyMixes 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.

FastGene® Taq ReadyMix PCR Kit

Technical Data Sheet

FastGene® Taq PCR Protocol

FastGene® Taq DNA Polymerase can be used to replace any commercial Taq DNA polymerase in an existing protocol. Due to differences in buffer formulations, it may be necessary to re-optimize annealing temperatures using FastGene® Taq ReadyMix should existing annealing temperatures give poor results.

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
2X FastGene® Taq ReadyMix (1.5 mM $MgCl_2$ at 1X)[2]	25 µl	1X
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[3]	As required	As required

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

[2] 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.

[3] ≤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
Denaturation	95 °C	30 sec	35[3]
Annealing[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:
info@nippongenetics.eu