NIPPON Genetics EUROPE GmbH

FastGene® Taq ReadyMix PCR Kit

Technical Data Sheet

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

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 (2x) 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.

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.

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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. 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 ºC	3 min[1]	1
Denaturation	95 ºC	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/

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