FastGene® Optima HotStart ReadyMix

Technical Data Sheet

Product Description

The FastGene® Optima PCR system is a blend of Taq DNA polymerase and an engineered archaeal (B-family) DNA polymerase. Both enzymes possess 5′ – 3′ polymerase activity, but only Taq possesses 5′ – 3′ exonuclease activity, and only the B-family DNA polymerase possesses 3′ – 5′ exonuclease activity. This two-enzyme system is designed to support robust, long-range, and sensitive PCR.

In the HotStart formulation, the enzyme blend is combined with a proprietary antibody that inactivates the enzyme until the first denaturation step. This eliminates spurious amplification products resulting from non-specific priming events during reaction setup and initiation, and increases overall reaction efficiency.

FastGene® Optima HotStart ReadyMix with dye (2X) is a ready to-use cocktail containing all components for a standard to long-range PCR, except primers and template. The 2X ReadyMix contains FastGene® Optima DNA Polymerase blend (0.2 U per µl reaction), FastGene® Optima Buffer (1X), dNTPs (0.4 mM of each dNTP at 1X), $MgCl_2$ (4 mM at 1X) and stabilizers.

The FastGene® Optima 2X ReadyMix also 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

The FastGene® Optima (HotStart ReadyMix kit with dye) is ideally suited for:

- Routine PCR
- PCR using complex templates
- SNP Analysis
- Any standard PCR application for which a hot start formulation of a high-quality thermostable DNA polymerase is required.

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.

Kit Codes and Components		
LS29	FastGene® Optima HotStart ReadyMix	5 x 1.25 ml (500 rxns)
LS31	FastGene® Optima HotStart ReadyMix	250 µl (20 rxns)
Related Products		
LS20	FastGene® Taq DNA Polymerase	100 Units
LS21	FastGene® Taq DNA Polymerase	500 Units
LS22	FastGene® Taq DNA Polymerase	2000 Units
LS23	FastGene® HotStart TAQ DNA Polymerase	100 Units
LS24	FastGene® HotStart TAQ DNA Polymerase	250 Units
LS25	FastGene® HotStart TAQ DNA Polymerase	1000 Units
LS26	FastGene® TAQ Ready Mix PCR Kit	50 x 50µl rxns
LS27	FastGene® TAQ Ready Mix PCR Kit	250 x 50µl rxns
LS28	FastGene® Optima	250 Units
LS30	FastGene® Optima	100 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

- Denature at 94°C, increase denaturation times for fast-block instruments.
- Extend at 68°C, especially for long range/high sensitivity PCR.
- ReadyMix is supplied at 2x concentration.
- Products can be cloned in T-overhang cloning vectors.

Shipping and Storage

FastGene® Optima HotStart ReadyMix with dye 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.

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FastGene® Optima Protocol

FastGene® Optima HotStart ReadyMix DNA Polymerase blend can be used to replace any commercial (hot start) Taq DNA polymerase in an existing protocol.

Step 1a: 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:

Step 1b: Prepare the PCR master mix

Component	25 µl rxn	Final conc.
PCR-grade water	Up to 25 µl	N/A
2X FastGene® Optima HotStart ReadyMix with dye	12.5 µl	1X
Forward Primer (10 µM)	1.25 µl	0.5 µM
Reverse Primer (10 µM)	1.25 µl	0.5 µM
Template DNA[1]	As required	As required

[1] ≤100 ng for genomic DNA; ≤1 ng for less complex DNA (e.g. plasmid, lambda).

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	15 sec	20-40[4]
Annealing[2]	T_m – 5 °C	15 - 30 sec	
Extension[3]	72 °C	1 min/kb	
Final extension (optional)[5]	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] An extension temperature of 68 °C is recommended for long-range (5 kb to 10 kb) PCR products.

[4] 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.

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

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