

# 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<sub>2</sub> (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 LS31	FastGene® Optima HotStart ReadyMix FastGene® Optima HotStart ReadyMix	5 x 1.25 ml (500 rxns) 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		
<ul style="list-style-type: none"> <li>• Denature at 94°C, increase denaturation times for fast-block instruments.</li> <li>• Extend at 68°C, especially for long range/high sensitivity PCR.</li> <li>• ReadyMix is supplied at 2x concentration.</li> <li>• Products can be cloned in T-overhang cloning vectors.</li> </ul>		

### 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 <sup>1</sup>	As required	As required

<sup>1</sup> ≤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 <sup>1</sup>	1
Denaturation	95 °C	15 sec	20-40 <sup>4</sup>
Annealing <sup>2</sup>	$T_m - 5$ °C	15 - 30 sec	
Extension <sup>3</sup>	72 °C	1 min/kb	
Final extension (optional) <sup>5</sup>	72 °C	1 min/kb	1
Store	4 – 10 °C	HOLD	1

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> An extension temperature of 68 °C is recommended for long-range (5 kb to 10 kb) PCR products.

<sup>4</sup> 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.

<sup>5</sup> 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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