FastGene® HiFi HS 2x Master Mix

Manual (LS36)

Product description

The FastGene® HiFi PCR mix contains an engineered version of the Pfu DNA polymerase. The enzyme possesses a 3'-5' exonuclease and a proof-reading activity. Furthermore, the FastGene® HiFi HS polymerase has a significantly improved DNA binding activity and processivity compared to standard Pfu. This results in faster extension, higher yields and the robust amplification of difficult targets.
The enzyme is combined with an aptamer-like molecule that inhibits the enzyme until the first denaturation step. This eliminates primer dimer formation and amplification of non-specific products, augmenting specificity and sensitivity of the PCR reaction. Thereby, the FastGene® HS HiFi PCR mix is highly suitable for multiplexing and applications where high accuracy is needed, such as cloning, site-directed mutagenesis and sequencing.
FastGene® HiFi HS 2x Master Mix is a ready-to-use cocktail containing an advanced buffer system with dNTPs, Mg2+, enhancers as well as the FastGene® HiFi HS polymerase. Thus, the FastGene® HiFi HS 2x Master Mix includes all components necessary for a wide range of targets and fragment sizes, regardless of GC contents. PCR products amplified with this mix are blunt ended.

Product applications

The FastGene® HiFi HS 2x Master Mix is ideally suited for:

- PCR using complex templates
- Cloning
- Site-directed mutagenesis
- Sequencing
- Multiplexing
- Routine PCR
- Any standard PCR application for which a hot start formulation of a high-quality thermostable DNA polymerase is required

Kit Codes and components		
LS36	FastGene® HiFi HS 2x Master Mix	2x 1.25 ml, 100 rxns (50 µl)
LS36s	FastGene® HiFi HS 2x Master Mix (sample)	125 µl, 5 rxns (50 µl)

Quick notes

Denautre at 95 °C
Extend at 72 °C
Ready Master Mix is supplied at 2x concentration

Shipping and storage

FastGene® HiFi HS 2x Master Mix 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.

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 components

FastGene® HiFi HS 2x Master Mix.

FastGene® HiFi HS 2x Master Mix protocol

FastGene® HiFi HS 2x Master Mix DNA Polymerase blend can be used to replace any commercial (hot start) HiFi DNA polymerase in an existing protocol.

Step 1: Reaction Setup

- 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:

Reagent	25 µl rxn	50 µl rxn	Final conc.
FastGene® HiFi HS 2x Master Mix[1]	12.5 µl	25 µl	1x
Forward primer (10 µM)[2]	1.0 µl	2.0 µl	400 nM
Reverse primer (10 µM)[2]	1.0 µl	2.0 µl	400 nM
Template DNA	As required[3]	As required[4]	variable
PCR-grade water	Up to 25 µl	Up to 50 µl	---

[1] The 2x mix contains FastGene® HiFi HS polymerase, 6 mM MgCl2, 2 mM dNTPs, enhancers and stabilizers. It is not recommended to add further PCR enhancers or MgCl2 to the reaction. The mix composition has been optimized to maximize PCR success rates

[2] Primers should have a predicted melting temperature of around 60°C. The final primer concentration in the reaction should be between 0.2 µM and 0.6 µM.

[3] <100 ng genomic DNA; <5 ng less complex DNA (e.g. plasmid, lambda)

[4] <200 ng genomic DNA;<10 ng less complex DNA (e.g. plasmid, lambda)

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

Step 2: Reaction Setup

- Run the PCR with the following cycling protocol:

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	1 min	1 x
Denaturation[5]	95 °C	15 sec	25-35 x
Annealing[6]	60 °C - 75 °C	15 sec	
Extension[7]	72 °C	30 sec/kb	

[5] Denaturation should be performed at 95°C. However, at presence of high GC regions increasing the melting temperature to 98-100°C can improve the total yield.

[6] An annealing temperature of 60°C 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.

[7] Best extension is achieved at 72°C. However, optimal extension time is dependent on amplicon length and complexity of template. 30 seconds per kilobase (kb) is recommended for most applications. However shorter extension times between 10 and 30 seconds per kb are feasible. Two-step cycling protocols may also be used with combined annealing and extension at 68-75°C. If using shorter extension times, loading too much template DNA must be prevented. If non-specific bands are visible after amplification, the amount of template DNA should be decreased.

NIPPON Genetics EUROPE GmbH

+49 2421 55496 0
+49 2421 55496 11
info@nippongenetics.eu
www.nippongenetics.eu