FastGene® apTaq HotStart Polymerase

Technical Data Sheet

Product Description

The FastGene® apTaq HotStart Polymerase uses an aptamer based HotStart activation technology. The aptamer based HotStart inhibits any polymerase acitivity at temperatures below 45 °C and has an immediate activation.

Product Applications

The FastGene® apTaq HotStart Polymerase is ideally suited for:

- Fast PCR
- 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.

Shipping and Storage

FastGene® apTaq HotStart Polymerase 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. Please make sure to centrifuge briefly each tube prior usage.

Kit Codes and Components

LS34s	**FastGene® apTaq HotStart Polymerase**	**Sample**
LS34	**FastGene® apTaq HotStart Polymerase**	**500 Units**

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
LS29	FastGene® Optima HotStart ReadyMix	500 x 25 µl rxns
LS30	FastGene® Optima	50 Units
LS31	FastGene® Optima HotStart ReadyMix	20 x 25 µl rxns

Direct PCR

LS05	DNAreleasy Advance	10 preps
LS06	DNAreleasy Advance	50 preps

Quick Notes

- Replace Taq for Standard PCR, with no protocol change.
- Buffer is supplied at 5x concentration with separate magnesium for optimization.
- Products can be cloned in T-overhang cloning vectors.

Optimizing $MgCl_2$

Aptamer-based HotStart polymerase and/or reactions with increased amounts of template (e.g., genomic DNA), primer and nucleotides might require higher $MgCl_2$ concentrations. A separate 50 mM $MgCl_2$ solution is supplied with the enzyme. Please adjust the $MgCl_2$ concentration according to the table below:

Final conc. in 50 µl reaction	2 mM	2.5 mM	3 mM	3.5 mM	4 Mm
Volume of 50mM $MgCl_2$ Stock	2 µl	2.5 µl	3 µl	3.5 µl	4 µl

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FastGene® apTaq Protocol

FastGene® apTaq HotStart Polymerase can be used to replace any commercial Taq DNA polymerase in an existing protocol. To allow the most seamless integration of FastGene® apTaq HotStart Polymerase into existing protocols, be sure to match reaction conditions, particularly the $MgCl_2$, primer and enzyme concentrations, as closely as possible.

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
5 U/µl of FastGene® apTaq	0.5 µl[2]	2.5 U[2]
5X FastGene® apTaq Buffer	10 µl	1X
50 mM $MgCl_2$	2 µl[3]	2 mM[3]
dNTP Mix (10 mM each)	1 µl	0.2 mM each
Forward Primer (10 µM)	2.5 µl	0.5 µM
Reverse Primer (10 µM)	2.5 µl	0.5 µM
Template DNA[4]	As required	As required

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

[2] For small to midrange products, half the concentration of enzyme can be used. For GC-rich and other difficult templates, higher enzyme concentrations (up to 5 U per 50 µl reaction) may be required.

[3] A final $MgCl_2$ concentration of 2 mM is sufficient for most standard applications. For assays that do not perform well with 2 mM $MgCl_2$, the optimal $MgCl_2$ concentration for each primer–template combination should be determined empirically.

[4] ≤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

Step	Temperature	Duration	Cycles
Initial denaturation	95 °C	1 min[1]	1
Denaturation	95 °C	30 sec	20-40[3]
Annealing[2]	T_m + 2 °C	15-30 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 1 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 2 °C higher 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 (gradient should be at least between -2°C and +3°C).

[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:

https://www.nippongenetics.eu/en/terms-and-conditions/

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