i-StarMAX II DNA Polymerase [for Long & Hot Start PCR]

Cat. No. 25173 250 Units

DESCRIPTION

i-StarMAX™ II DNA Polymerase is an optimal enzyme mixture of *i*-StarTaq™ DNA Polymerase and a proofreading DNA polymerases. *i*-StarTaq™ DNA Polymerase is a thermostable, modified form of recombinant *Taq* DNA Polymerase suitable for hot start PCR experiments. As a result of addition of *i*-StarTaq™ DNA Polymerase, *i*-StarMAX™ II DNA Polymerase cannot be useful tool only in amplification of short and long fragments but also problematic template/primer systems.

High yields of PCR product can be achieved using extension times as short as from 30 seconds to 1 minute per kb per cycle with the *i*-StarMAX™ II DNA Polymerase. The *i*-StarMAX™ II DNA Polymerase is recommended for relatively rapid, high-fidelity amplification of PCR targets up to 20kb when proofreading DNA polymerase alone requires too long an extension time or yields are insufficient.

STORAGE

Store at -20℃.

CHARACTERISTICS

- High fidelity, specificity, and yields
- Versatile for various DNA template including cloned fragment, phage DNA, mammalian genomic DNA and etc.
- Ideal for difficult templates, such as GC-rich or looped sequences

APPLICATIONS

- Amplification of genomic DNA and cDNA targets up to 20kb long with high specificity, sensitivity, and yield.
- PCR with difficult template/primer system.
- Multi-plex PCR
- Cloning with TA and blunt ends.

KIT CONTENTS

Label	25173 (250 Units)
***i*-StarMAX II DNA Polymerase (5U/㎕)**	250 Units
10X PCR Buffer* (w/20mM Mg^{2+})	1.5 ml
10X Mg^{2+} free PCR Buffer	1.5 ml
10mM dNTPs (2.5mM/each)	800 ㎕
25mM Mg^{2+}	1.5 ml

*** 10× PCR BUFFER,** 300 mM Tris-HCl(pH 9.3); 300 mM salts containing of K^+ and NH_4^+; 20 mM Mg^{2+}; Enhancer solution

GENERAL REACTION MIXTURE for PCR (total 20㎕)

Template	1 ng-1 ㎍
Primer (F)	5-10 pmoles
Primer (R)	5-10 pmoles
i-StarMAX™ II DNA polymerase (5U/㎕)	0.25-0.5 ㎕
10x PCR buffer	2 ㎕
dNTP Mixture (2.5mM each)	2 ㎕
Sterilized distilled water	up to 20 ㎕

CYCLING STEPS for SHORT and LONG FRAGMENTS

Cycle program for fragments < 10kb

	Temp	Time	Cycle No.
Initial Denaturation	92-94℃	2-4min	1
Denaturation Annealing Extension*	94℃ 45-65℃ 72℃	15s-1min 15s-1min 1min/1-1.5kb	25-30
Final extension	72℃ 4℃	5-10min hold	1

*, Extension time for 30s-1min is sufficient for fragments < 1kb.

Cycle program for fragments > 10kb

	Temp	Time	Cycle No.
Initial Denaturation	92-94℃	2-4min	1
Denaturation Annealing Extension	94℃ 45-65℃ 72℃	15s-1min 15s-1min 1min/1-1.5kb	10
Denaturation Annealing Extension	94℃ 45-65℃ 72℃	15s-1min 15s-1min 1min/1-1.5kb + 20s/cycle	15-20
Final extension	72℃ 4℃	5-10min hold	1

Note : This "SUGGESTED CYCLING PARAMETERS" serves as a guideline for PCR amplification. Optimal reaction conditions such as PCR cycles, annealing temperature, extension temperature and incubation times, may vary and must be individually determined.

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

• ***Amplification of various Hot-Start PCR condition.***

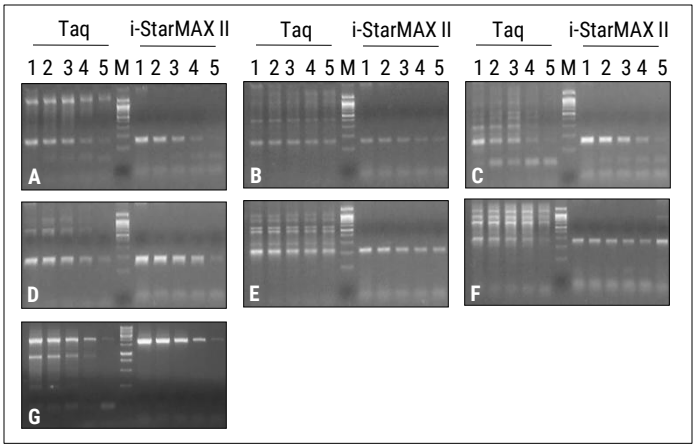

Fig 1. Amplification of various Hot-Start PCR condition.
[A] 161 bp amplification; **[B]** 165 bp amplification; **[C]** 166 bp amplification; **[D]** 181 bp amplification; **[E]** 218 bp amplification; **[F]** 266 bp amplification; **[G]** 1.8 Kb amplification
Lane M, 100bp Ladder DNA Marker; **lane a**, 100ng genomic DNA; **lane b**, 5^{-1} diluted genomic DNA ; **lane c**, 5^{-2} diluted genomic DNA; **lane d**, 5^{-3} diluted genomic DNA; **lane e**, 5^{-4} diluted genomic DNA

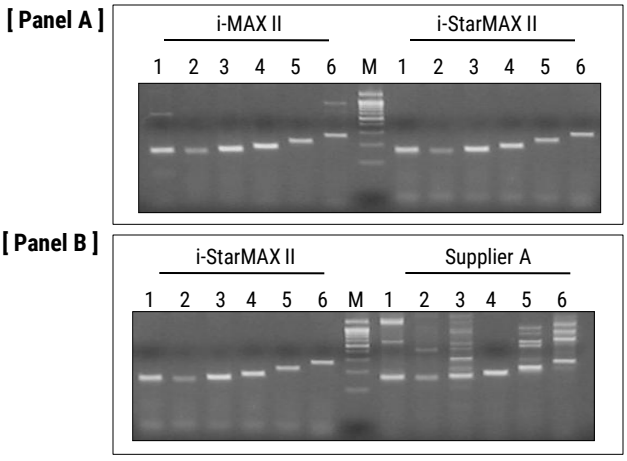

Fig 2. Amplification of various Hot-Start PCR condition.
[Panel A] Comparison of PCR amplification with *i*-Max II DNA polymerase
[Panel B] Comparison of PCR amplification with supplier A
Lane M, 100bp Ladder DNA Marker; **lane 1**, 161bp; **lane 2**, 165bp; **lane 3**, 166bp; **Lane 4**, 181bp; **lane 5**, 218bp; **lane 6**, 266bp

• ***Amplification of LA PCR Result***

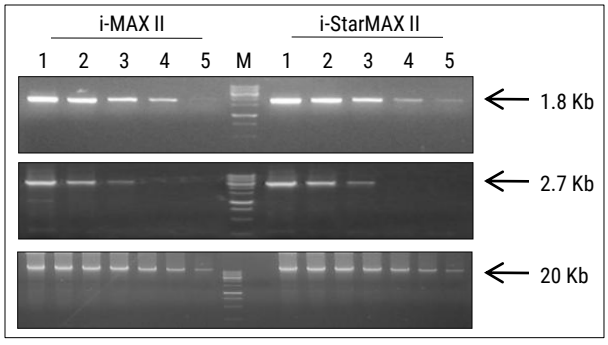

Fig 3. Amplification of LA PCR Result
Comparison of PCR amplification with *i*-MAX II DNA Polymerase

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Little or no PCR product	Primer problems due to - not optimal design - concentration - too high annealing temperature	- Design alternative primers - Reduce annealing temperature - Use primer of 5-20pmoles per 20㎕ reaction. - If you use an established primer pair, check performance on an established PCR system (control template).
	Enzyme concentration too low	- Use 0.1-2.5U of *i-StarMAX*™ II DNA polymerase per 20㎕ reaction. - If nessary, increase the amount of polymerase in 0.5U steps.
Multiple bands or background smear	Annealing temperature too low	- Increase annealing temperature in 2℃ steps.
	Primer design or concentration not optimal	- Review primer design - Titrate primer concentration
	Too high starting concentration of Mg-ions, template, cycles, or enzyme	- Reduce one or all of the contents.
Specific problems in RT-PCR application: no product, additional bands, background smear		- The volume of cDNA template (RT-reaction) should not exceed 10% of the final concentration of the PCR reaction. - Titrate cDNA template. - Follow trouble shooting above.

RELATED PRODUCTS

Product Name	Cat. No.
G-spin™ Total DNA Extraction Mini Kit	17045/17046
MEGAquick-spin™ Total Fragment DNA Purification Kit	17286 / 17287 / 17288
DNA-spin™ Plasmid DNA Purification Kit	17096 / 17097 / 17098
Maxime RT PreMix Kit (Oligo[dT]$_{15}$ Primer)	25081
Maxime RT PreMix Kit (Random Primer)	25082
Maxime PCR PreMix Kit (i-StarMAX II)	25281
RevoScript™ RT PreMix Kit(Random Primer)	25085 / 25086
RevoScript™ RT PreMix Kit(Oligo dT$_{15}$ Primer)	25083 / 25084
RealMOD™ Real-time PCR Master mix Kit(2X)	25341 / 25342
RealMOD™ Green Real-time PCR Master mix Kit(2X)	25343 / 25344