For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

ISO 9001/14001 Certified Company

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

Cat. No. INT-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 15kb when proofreading DNA polymerase alone requires too long an extension time or yields are insufficient.

STORAGE

Store at -20℃, and then stable for at least one year.

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 15kb long with high specificity, sensitivity, and yield.
- PCR with difficult template/primer system.
- Multi-plex PCR
- Cloning with TA and blunt ends.

KIT CONTENTS

• *i-StarMAX*™ II DNA Polymerase	50 units (5U/μl)
• 10x PCR buffer (20mM Mg^{2+})	1.5ml
• 10x Mg^{2+} free buffer	1.5ml
• 10mM dNTPs (2.5mM each)	800μl
• 25mM Mg^{2+}	1.5ml

10x PCR BUFFER

- 300mM Tris-HCl (pH9.3)
- 300mM Salts consisting of K^+ and NH_4^+
- 20mM Mg^{2+}

GENERAL REACTION MIXTURE for PCR (total 20μl)

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

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

Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

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

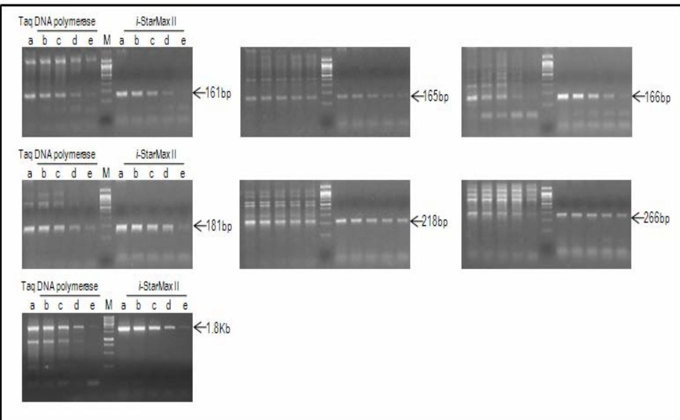
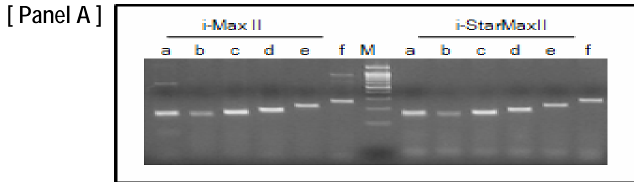


Fig 1. *Amplification of various Hot-Start PCR condition.*
Lane M, 100bp Ladder DNA Marker; **lane a**, 100ng genomic DNA; **lane b**, 5^{-1} diluted genomic DNA ; **lane c**, 5^{-2} diluted genomic DNA; **lane d**, 5^{-3} diluted genomic DNA; **lane e**, 5^{-4} diluted genomic DNA

[Panel A]



[Panel B]

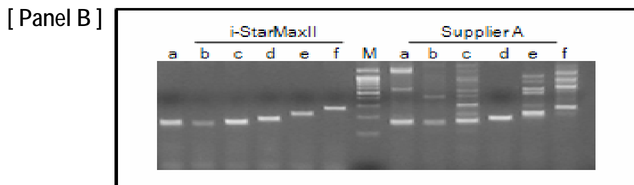


Fig 2. *Amplification of various Hot-Start PCR condition.*
Lane M, 100bp Ladder DNA Marker; **lane a**, 161bp; **lane b**, 165bp; **lane c**, 166bp; **lane d**, 181bp; **lane e**, 218bp; **lane f**, 266bp
[Panel A] Comparison of PCR amplification with *i*-Max II DNA polymerase
[Panel B] Comparison of PCR amplification with supplier A

• *Amplification of LA PCR Result*

[Panel A] [Panel B]

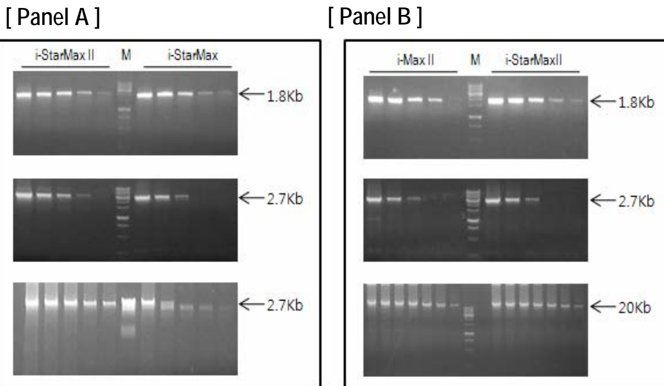


Fig 3. *Amplification of LA PCR Result*
[Panel A] Comparison of PCR amplification with *i*-StarMax DNA polymerase
[Panel B] Comparison of PCR amplification with *i*-Max II DNA polymerase

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*™ 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.
i-genomic CTB DNA Extraction Kit	17341
i-genomic Blood DNA Extraction Kit	17351
i-genomic BYF DNA Extraction Kit	17361
i-genomic Plant DNA Extraction Kit	17371
DNA-spin™ Plasmid DNA Extraction Kit	17091 / 17093
Power cDNA Synthesis Kit	25011 / 25012
100bp / 1Kb Ladder DNA Marker	24012 / 24022
i-StarTaq DNA polymerase	25161 / 25162