ISO 9001/14001 Certified Company

i-MAX II DNA Polymerase [for Enhancing PCR]

Cat. No. 25261 250 Units

DESCRIPTION

Taq DNA Polymerase is the most common PCR enzyme for amplifying up to 10kb lDNA templates and up to 3kb genomic templates. However, it is not able to amplify even longer DNA fragments. To overcome this constraint, variable PCR systems have been developed by various companies, which are containing *Taq* DNA Polymerase and thermostable DNA Polymerase with proofreading activity. Both *i*-MAX™ and *i*-MAX™ II DNA Polymerase are the PCR System which are developed for amplifying long and complex fragments. The first is designed for amplification of 5 10kb fragments from genomic DNA. The second (*i*-MAX™ II DNA Polymerase) can amplify even longer fragments up to 20kb from human genomic DNA, and up to 30kb from a lDNA template). Moreover, the second have improved amplification efficiency compared to *i*-MAX™ DNA Polymerase by improving enzyme activity. Therefore *i*-MAX™ II DNA Polymerase is a more versatile enzyme blend than *i*-MAX™ DNA Polymerase in amplifying various templates including short and long DNA fragment or simple and complex DNA, either.

STORAGE

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

CHARACTERISTICS

- Increased fidelity of PCR amplification, because the *i*-MAX™ II DNA Polymerase enzyme blend combines the proofreading activity of *Pfu* DNA Polymerase with the high processivity of *Taq* DNA Polymerase.
- Increased yield of PCR amplification, because the accuracy of the enzyme blend reduces the number of truncated amplification products formed.
- Improved performance of long PCR, because the reaction buffer and the enzyme blend are optimized for generation of certain length products.

APPLICATIONS

- Standard and long PCR
- PCR with difficult templates
- Cloning with TA and blunt ends

KIT CONTENTS

Label	25261 (250 Units)
***i*-MAX 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 9.0); 300 mM salts containing of K^+ and NH_4^+; 20 mM Mg^{2+}; Enhancer solution

GENERAL REACTION MIXTURE for PCR (total 20㎕ reaction)

Template	1pg-1㎍
Primer 1	5-10 pmoles
Primer 2	5-10 pmoles
i-MAX™ 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 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.

PCR OPTIMIZATION

To produce high yields of specific DNA target sequences, individual reaction component concentrations (and time and temperature parameters) must be adjusted within suggested ranges for efficient amplification of specific targets.

Template DNA

For amplifying long target fragment, it is important that you use high quality, intact, and high pure template DNA.

Primers

PCR primers are oligonucleotide, typically 15-30 bases long, hybridizing to opposite strands and flanking the region of interest in the target DNA. A 40%-60% G+C content is recommended for both primers.

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

• Amplification

1) Amplification of 570bp Fragment

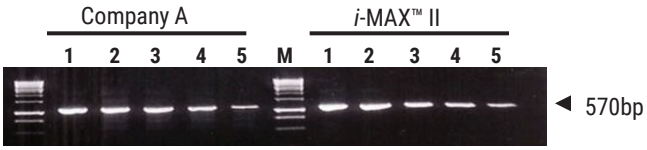


Figure 1. Comparison of *i*-MAX™ II DNA Polymerase and Company A's PCR System by amplifying 570bp DNA fragment (GAPDH).

Comparison of *i*-MAX™ II DNA Polymerase and Company A's PCR System by amplifying 570bp DNA fragment from variable amounts of cDNA Aliquots of 5㎕ in 20㎕ reaction are loaded on 8% agarose gel. The *i*-MAX™ II DNA Polymerase have comparable sensitivity and amplifying performance with company A's long-range PCR System in amplifying 570bp DNA fragment. **Lanes M,** 100bp Ladder DNA marker (iNtRON's); **lanes 1,** 2^{-3} diluted cDNA; **lane 2,** 2^{-4} diluted cDNA; **lane 3,** 2^{-5} diluted cDNA; **lane 4,** 2^{-6} diluted cDNA; **lane 5,** 2^{-7} diluted cDNA

2) Amplification of 1.3Kb Fragment

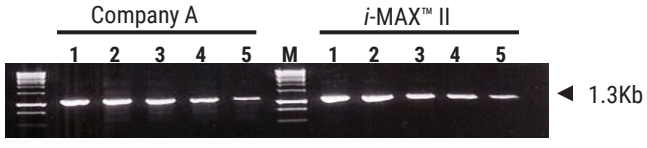
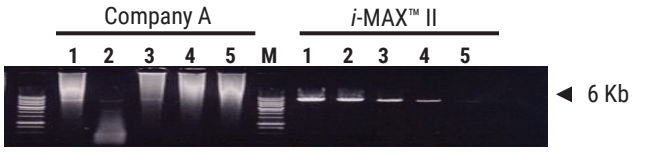


Figure 2. Comparison of *i*-MAX™ II DNA Polymerase and Company A's PCR System by amplifying 1.3 kb DNA fragment.

Comparison of *i*-MAX™ II DNA Polymerase and Company A's PCR System by amplifying 1.3 kb DNA fragment from variable amounts of human genomic DNA Aliquots of 5㎕ in 20㎕ reaction are loaded on 8% agarose gel. The *i*-MAX™ II DNA Polymerase have comparable sensitivity and amplifying performance with company A's long-range PCR System in amplifying 1.3kb DNA fragment. **Lanes M,** 1kb Ladder DNA marker (iNtRON's); **lanes 1,** 300 fg; **lane 2,** 150 fg; **lane 3,** 75fg; **lane 4,** 37.5fg; **lane 5,** 18.75fg

3) Amplification of 6Kb / 20Kb Fragment

[Panel A]



[Panel B]

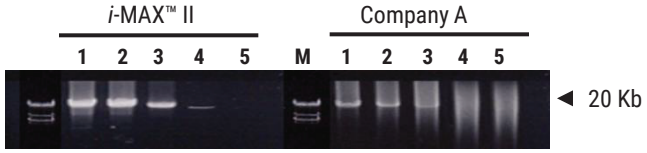


Figure. 3. Comparison of *i*-MAX™ II DNA Polymerase and Competitor A's PCR System in amplifying 6kb and 20kb fragment

Comparison of *i*-MAX™ II DNA Polymerase and Company A's PCR System by amplifying 6Kb, 20Kb DNA fragment from variable amounts of λDNA. Aliquots of 5㎕ in 20㎕ reaction are loaded on 8% agarose gel. The *i*-MAX™ II DNA Polymerase have comparable sensitivity and amplifying performance with company A's long-range PCR System in amplifying 6Kb, 20Kb DNA fragment. **[Pannel A]** 6 Kb product; **[Pannel B]** 20kb product

[Pannel A] Lanes M, λDNA *Eco*RI digest; **lanes 1,** 300 fg; **lane 2,** 150 fg; **lane 3,** 75 fg; **lane 4,** 37.5 fg; **lane 5,** 18.75 fg

[Pannel B] Lanes M, λDNA *Eco*RI digest; **lanes 1,** 1 ng; **lane 2,** 100 pg; **lane 3,** 10 pg; **lane 4,** 1 pg; **lane 5,** 100 fg

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Little or no product	Primer	- Primer concentration not optimal or primers degraded or primers degraded
	Problems with starting template	- Check the concentrations, storage conditions, and quality of the starting template.
	Incorrect annealing temperature or time	-Decrease annealing temperature by 2℃ increments.
	Extension time too short	- Annealing time should be between 30 and 60s.
	Hot start may be necessay	-Increase the extension time by increments of 1min.
Product is multi-banded or smeared		-Perform manual hotstrat PCR or iNtRON's hotstart PCR enzyme (*i*-StarMAX™).
	Annealing temperature too low	-Including the points mentioned above, check the facts below.
	Hot start may be necessary	-Increase annealing temperature in 2℃ increments.
	Primer design not optimal	
	Too much start template	