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

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

ISO 9001/14001 Certified

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 λDNA 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 company, which are containing *Taq* DNA Polymerase and thermostable DNA Polymerase with proofreading activity. *i*-MAX™ and *i*-MAX™ II DNA Polymerase both also 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 λDNA 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 template 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

-*i*-MAX™ II DNA Polymerase(5 U/μl)	250 units
-10x PCR Buffer (20mM Mg^{2+})	1.5ml
-10x Mg^{2+} free Buffer	1.5ml
-10mM dNTPs (2.5mM each)	800 μl
-25mM Mg^{2+}	1.5ml

10x PCR BUFFER

-300mM Tris-HCl (pH9.0)
-300mM Salts consisting of K^+ and NH_4^+
-20mM Mg^{2+}

GENERAL REACTION MIXTURE for PCR (total 20μl)

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

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.