

i-StarTaq™ DNA Polymerase [for Hot-Start PCR]

Cat. No. 25161 250 units

Cat. No. 25162 500 units

DESCRIPTION

The polymerase chain reaction (PCR) is a powerful technique designed to amplify high levels of DNA fragments from target DNA. However, inherent in the amplification power of PCR is the potential for contamination through co-amplification of nonspecific products, especially when the target comprises Abundant nontemplate DNA such as genomic DNA.

Hot start PCR technique was developed as a method to minimize the deleterious effects of mispriming at lower temperatures during PCR. In a PCR reaction, even short incubations at temperatures below the optimum annealing temperature for a particular set of primers can result in mispriming, elongation and the subsequent formation of spurious bands. The Hot Start technique involves inactivating (or leaving out) one critical component of the PCR reaction until the temperature has risen above this optimal annealing temperature.

iNtRON has developed a recombinant *Taq* DNA polymerase which is inactive below an annealing temperature, but can be activated above the annealing temperature. Therefore, iNtRON's *i-StarTaq*™ DNA Polymerase provides a solution for problematic template/primer PCR systems.

STORAGE

Store at -20 °C.

CHARACTERISTICS

- Sensitivity : reduced or no amplification of non-specific products resulting from mispriming during PCR.
- Specificity : generating fragments of high specificity and high yield.
- Flexibility : available for various DNA template including cloned fragment, phage DNA, mammalian genomic DNA and etc.

APPLICATIONS

- Amplification of genomic DNA and cDNA targets up to 5kb long with high specificity, sensitivity, and yield.
- PCR with difficult templates e.g. secondary structures or GC-rich sequences.
- Cloning with TA and blunt ends.

KIT CONTENTS

Label	25161 (250 Units)	25162 (500 Units)
i-StarTaq™ DNA Polymerase (5U/μℓ)	250 Units	500 Units
10X PCR Buffer* (w/20mM Mg ²⁺)	1 ml	1 ml
10X Mg²⁺ free PCR Buffer	1 ml	1 ml
10mM dNTPs (2.5mM/each)	500 μℓ	1 ml
25mM Mg²⁺	1 ml	1 ml

* 10x PCR BUFFER, 300 mM Tris-HCl(pH 9.0); 300 mM salts containing of K⁺ and NH₄⁺; 20 mM Mg²⁺; Enhancer solution

GENERAL REACTION MIXTURE for PCR (total 20μℓ reaction)

Template	1ng-1 μg
Primer 1	5-10 pmoles
Primer 2	5-10 pmoles
i-StarTaq™ DNA Polymerase (5U/μℓ)	0.2-0.5 μℓ
10x PCR buffer	2 μℓ
dNTP Mixture (2.5mM each)	2 μℓ
Sterilized distilled water	up to 20 μℓ

SUGGESTED CYCLING PARAMETERS

PCR cycle	Temp.	PCR product size			
		100-500bp	500-1000bp	1Kb-5Kb	
Initial denaturation	94 °C	2min	2min	2min	
30-40 Cycles	Denaturation	94 °C	20sec	20sec	20sec
	Annealing	50-65 °C	10sec	10sec	20sec
	Extension	65-72 °C	20-30sec	40-50sec	1min/Kb
Final extension	72 °C	Optional. Normally, 2-5min			

Note 1 : 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.



TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

• Comparison I with *i-Taq*TM DNA Polymerase

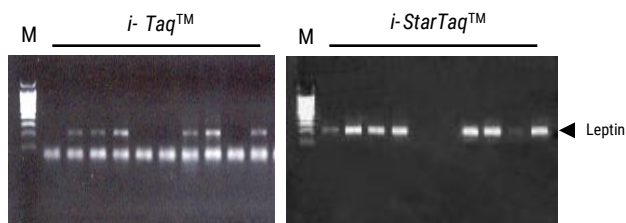


Fig. 1. PCR amplification of leptin gene from human clinical samples with *i-Taq*TM DNA Polymerase or *i-StarTaq*TM DNA Polymerase.

Total RNA was purified from human tissue samples using easy-BLUETM Total RNA Extraction Kit (Cat. No. 17061). And then leptin gene is amplified by RT-PCR using Power cDNA Synthesis Kit (Cat. No. 25011) and *i-Taq*TM DNA Polymerase or *i-StarTaq*TM DNA Polymerase. As shown in above figure, the specific fragment is amplified more efficiently and specifically with *i-StarTaq*TM DNA Polymerase than with *i-Taq*TM DNA Polymerase. In the case with *i-Taq*TM DNA Polymerase, the amplification of specific fragment is reduced by nonspecific amplification (eg, primer dimer). Whereas, in the case with *i-StarTaq*TM DNA Polymerase, the specific fragment is amplified more abundantly by reduced amplification of primer dimer. **Lane M**, 100bp Ladder DNA Marker; **lane 1-10**, amplicons of leptin from each individuals

• Comparison II with *i-Taq*TM DNA Polymerase

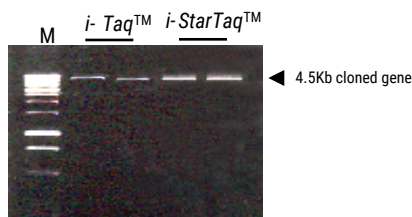


Fig. 2. Amplification of 4.5Kb cloned gene in vector using *i-Taq*TM DNA Polymerase or *i-StarTaq*TM DNA Polymerase.

*i-StarTaq*TM DNA Polymerase is more efficient in amplifying moderate long DNA fragments than common *i-Taq*TM DNA Polymerase. 8 μ l of the PCR products was loaded onto a 0.8% agarose gel. **Lane M**, 1Kb Ladder DNA Marker; **lane 1,2**, *i-Taq*TM; **lane 3,4**, *i-StarTaq*TM DNA Polymerase

• Comparison III with *i-Taq*TM DNA Polymerase

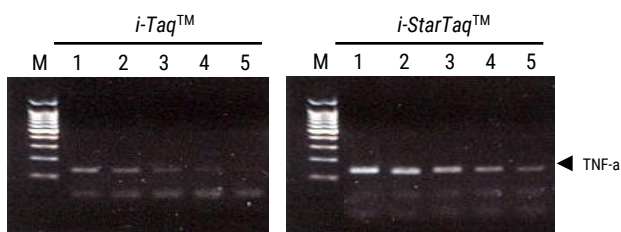


Fig. 3. PCR amplification of human TNF- α gene (131bp) with *i-Taq*TM DNA Polymerase or *i-StarTaq*TM DNA Polymerase

Genomic DNA was isolated with G-spinTM Genomic DNA Extraction Kit for Cell/Tissue (Cat.No. 17041) from human stomach cancer cell line, SNU-1. TNF- α gene was amplified with varying amounts of template. 8 μ l of the PCR products was loaded onto a 1.5% agarose gel.

Lane M, 100bp Ladder DNA Marker; **lane 1**, 100ng; **lane 2**, 50ng; **lane 3**, 25ng; **lane 4**, 12ng; **lane 5**, 6ng

• Comparison with antibody-blocked hot start enzyme

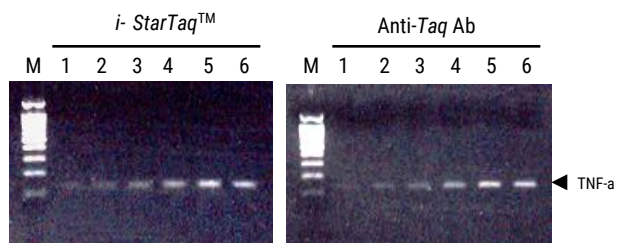


Fig. 4. PCR amplification of human TNF- α gene (131bp) with *i-StarTaq*TM DNA Polymerase or antibody-blocked hot start enzyme.

Genomic DNA was isolated with G-spinTM Genomic DNA Extraction Kit for Cell/Tissue (Cat.No. 17041) from human stomach cancer cell line, SNU-1. TNF- α gene was amplified with varying amounts of template. 8 μ l of the PCR products was loaded onto a 1.5% agarose gel.

Lane M, 100bp Ladder DNA Marker; **lane 1**, 3ng; **lane 2**, 6ng; **lane 3**, 12ng; **lane 4**, 25ng; **lane 5**, 50ng; **lane 6**, 100ng

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 μ l 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>i-StarTaq</i> TM DNA Polymerase per 20 μ l reaction. - If necessary, increase the amount of polymerase in 0.5U steps.
Multiple bands or background smear	Annealing temperature too low	- Increase annealing temperature in 2 $^{\circ}$ C 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>i-Taq</i> TM DNA Polymerase	25021 / 25022
<i>i-MAX</i> II DNA Polymerase	25261 / 25263
<i>i-StarMAX</i> TM II DNA Polymerase	25173 / 25179
RevoScript TM RT PreMix Kit(Random Primer)	25085 / 25086
RevoScript TM RT PreMix Kit(Oligo dT ₁₅ Primer)	25083 / 25084
RealMOD TM Real-time PCR Master mix Kit(2X)	25341 / 25342
RealMOD TM Green Real-time PCR Master mix Kit(2X)	25343 / 25344

