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

*ISO 9001/14001 Certified Company*

# *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 temerature. Therefore, iNtRON's *i-StarTaq*™ DNA Polymerase provides a solution for problematic template/primer PCR systems.

## STORAGE

Store at -20℃.

## 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/㎕)** | 250 Units | 500 Units |
| **10X PCR Buffer* (w/20mM $Mg^{2+}$ )** | 1 ml | 1 ml |
| **10X $Mg^{2+}$ free PCR Buffer** | 1 ml | 1 ml |
| **10mM dNTPs (2.5mM/each)** | 500 ㎕ | 1 ml |
| **25mM $Mg^{2+}$** | 1 ml | 1 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 | 1ng-1㎍ |
| Primer 1 | 5-10 pmoles |
| Primer 2 | 5-10 pmoles |
| *i*-StarTaq™ DNA Polymerase (5u/㎕) | 0.2-0.5㎕ |
| 10x PCR buffer | 2㎕ |
| dNTP Mixture (2.5mM each) | 2㎕ |
| Sterilized distilled water | up to 20㎕ |

## SUGGESTED CYCLING PARAMETERS

| PCR cycle | | Temp. | PCR product size | | |
|---|---|---|---|---|---|
| | | | 100-500bp | 500-1000bp | 1Kb-5Kb |
| Initial denaturation | | 94℃ | 2min | 2min | 2min |
| 30-40 Cycles | Denaturation | 94℃ | 20sec | 20sec | 20sec |
| | Annealing | 50-65℃ | 10sec | 10sec | 20sec |
| | Extension | 65-72℃ | 20-30sec | 40-50sec | 1min/Kb |
| Final extension | | 72 ℃ | 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™ DNA Polymerase

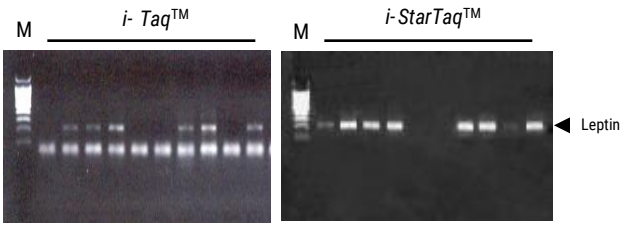


**Fig. 1. PCR amplification of leptin gene from human clinical samples with *i-Taq*™ DNA Polymerase or *i-StarTaq*™ DNA Polymerase.**

Total RNA was purified from human tissue samples using easy-BLUE™ 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*™ DNA Polymerase or *i-StarTaq*™ DNA Polymerase. As shown in above figure, the specific fragment is amplified more efficiently and specifically with *i-StarTaq*™ DNA Polymerase than with *i-Taq*™ DNA Polymerase. In the case with *i-Taq*™ DNA Polymerase, the amplification of specific fragment is reduced by nonspecific amplification (eg, primer dimer). Whereas, in the case with *i-StarTaq*™ 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™ DNA Polymerase

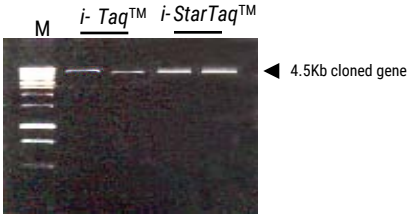


**Fig. 2. Amplification of 4.5Kb cloned gene in vector using *i-Taq*™ DNA Polymerase or *i-StarTaq*™ DNA Polymerase.**

*i-StarTaq*™ DNA Polymerase is more efficient in amplifying moderate long DNA fragments than common *i-Taq*™ DNA Polymerase. 8㎕ of the PCR products was loaded onto a 0.8% agarose gel.

**Lane M**, 1Kb Ladder DNA Marker; **lane 1,2,** *i-Taq*™; **lane 3,4,** *i-StarTaq*™ DNA Polymerase

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

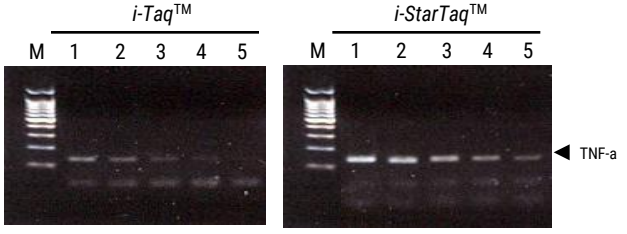


**Fig. 3. PCR amplification of human TNF-α gene (131bp) with *i-Taq*™ DNA Polymerase or *i-StarTaq*™ DNA Polymerase**

Genomic DNA was isolated with G-spin™ 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㎕ 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 anibody-blocked hot start enzyme

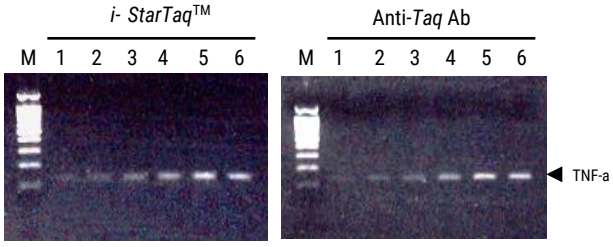


**Fig. 4. PCR amplification of human TNF-α gene (131bp) with *i--StarTaq*™ DNA Polymerase or anibody-blocked hot start enzyme.**

Genomic DNA was isolated with G-spin™ 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㎕ 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<br>- not optimal design<br>- concentration<br>- too high annealing temperature | - Design alternative primers<br>- Reduce annealing temperature<br>- Use primer of 5-20pmoles per 20㎕ reaction.<br>- 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-StartTaq*™ DNA Polymerase per 20㎕ reaction.<br>- 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<br>- 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.<br>- Titrate cDNA template.<br>- Follow trouble shooting above. |

## RELATED PRODUCTS

| Product Name | Cat. No. |
|---|---|
| *i*-Taq™ DNA Polymerase | 25021 /25022 |
| *i*-MAX II DNA Polymerase | 25261 / 25263 |
| *i*-StarMAX™ II DNA Polymerase | 25173 / 25179 |
| 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 |