# *i*-Taq™ DNA Polymerase

| | | |
|---|---|---|
| **Cat. No.** | **25021** | **250 units** |
| **Cat. No.** | **25022** | **500 units** |

## DESCRIPTION

PCR (polymerase chain reaction) was developed by *Kary Mullis* in mid 1980's and it has made development of modern molecular biology possible through DNA oligo sequence. The common usage of DNA polymerase in PCR method is *Taq* DNA polymerase. In the beginning, the enzyme used in PCR method was *E. coli* DNA polymerase, but enzyme had to be added at every step of the process due to its thermal instability.

Therefore, DNA polymerase was developed from *Thermus aquaticus* bacteria which thrives in hot spa. *Taq* DNA polymerase optimally compose DNA at 72 °C, therefore it could stably amplify a specified oligo sequence without adding enzyme at every due to its thermal stability even at 94 °C.

Purification process is most important step in making the enzyme: if it's not sufficiently purified, chromosomal DNA of *E.coli* or plasmid DNA cause contamination, and during PCR process, these DNA's are amplified instead of target DNA. Thus, to correct this problem, the iNtRON's *i-Taq*™ DNA Polymerase is developed.

## STORAGE

Store at -20°C.

## CHARACTERISTICS

- High efficiency of the amplification
- No DNA contamination grade enzyme
- High fidelity of PCR product
- Low price & rapid delivery
- Include dNTP

## APPLICATIONS

- Genomic DNA PCR
- RT-PCR
- Direct sequencing related PCR
- T/A vector cloning
- LOH or MSI analysis related PCR

## KIT CONTENTS

| Label | 25021 (250 Units) | 25022 (500 Units) |
|---|---|---|
| ***i*-Taq™ DNA Polymerase (5U/μl)** | 250 Units | 500 Units |
| **10X PCR Buffer* (w/20mM $MgCl_2$ )** | 1 ml | 1 ml |
| **10X $MgCl_2$ free PCR Buffer** | 1 ml | 1 ml |
| **10mM dNTPs (2.5mM/each)** | 500 μl | 1 ml |
| **25mM $MgCl_2$** | 1 ml | 1 ml |

*** 10× PCR BUFFER,** 100 mM Tris-HCl(pH 8.3); 500 mM KCl; 20 mM $MgCl_2$; Enhancer solution

## GENERAL REACTION MIXTURE for PCR (total 20μl)

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

## 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 :** 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

### • *Sensitivity*

Serial dilution of a template DNA were amplified using *i-Taq*™ DNA Polymerase in same amount polymerase. The PCR product has been identified up to 2pg genomic DNA.

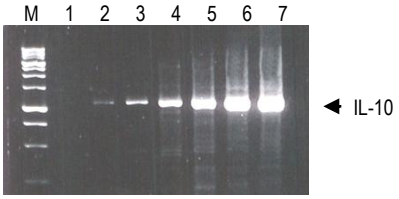


**Fig. 1. Sensitivity of the *i-Taq*™ DNA Polymerase**
We confirmed the sensitivity of amplification from serial dilution of the human genomic DNA(IL-10) using the *i-Taq*™ DNA Polymerase .Total volume is 20 ㎕ contained 1 unit *i-Taq*™ DNA Polymerase, it is analyzed on a agarose gel
**Lane M,** Marker DNA; **lane 1,** Negative control ; **lane 2,** 2pg template DNA; **lane 3,** 20pg template DNA; **lane 4,** 200pg template DNA; **lane 5,** 2ng template DNA; **lane 6,** 20ng template DNA; **lane 7,** 200ng template DNA

### • *Activity per Batch*

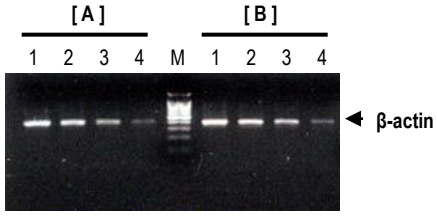


**Fig. 2. Amplification of beta-actin with *i-Taq*™ DNA Polymerase**
The first cDNA was synthesized from human cell and synthesized cDNA were 2-fold diluted to use templates for PCR. PCR reaction was performed with the beta-actin primer (400bp) using *i*-Taq Polymerase.
**Lane M,** Marker DNA; **lane 1,** $2^{-4}$ diluted *i-Taq*™ DNA Polymerase; **lane 2,** $2^{-5}$ diluted *Taq* DNA pol.; **lane 3,** $2^{-6}$ diluted *i-Taq*™ DNA Polymerase; **lane 4,** $2^{-7}$ diluted *i-Taq*™ DNA Polymerase.

### • *PCR amplification*

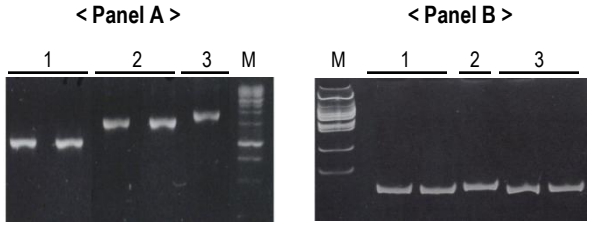


**Fig. 3. PCR amplification.**
Total genomic DNA of *Esherichia coli* was isolated with G-spin™ Genomic DNA Extraction Kit for Bacteria (Cat.No. 17121). Then the PCR reaction was performed using *i-Taq*™ DNA Polymerase (Cat. No. 25021) to specifically amplify class 1 integron gene in *E. coli* strains (Panel A) and class 1 integron promoter gene in *E. coli* strains (Panel B).
**< Panel A >**
**Lane M,** 1Kb Ladder DNA Marker; **lane 1,** 1.2kb size amplicon; **lane 2,** 1.6kb size amplicon; **lane 3,** 2.0kb size amplicon
**< Panel B >**
**Lane M,** 100bp Ladder DNA Marker; **lane 1, 3,** 80bp size amplicon; **lane 2,** 84bp size amplicon

### • *RT-PCR amplification*

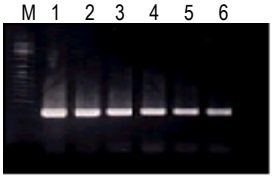


**Fig.4. RT-PCR Amplification at the Indicating cDNA diluted mixtures.**
Total RNA was purified from mouse cells using easy-BLUE™ Total RNA Extraction Kit (Cat. No. 17061). And then, the first strand of cDNA was synthesized using Power cDNA Synthesis Kit (Cat. No. 25011). After diluting the cDNA mixture as indicates, the RT-PCR reaction was performed with 2.5 units of *i-Taq*™ DNA Polymerase.
**Lane M,** 100bp Ladder DNA Marker; **lane 1,** undiluted cDNA; **lane 2,** 1/2 diluted cDNA; **lane 3,** 1/4 diluted cDNA; **lane 4,** 1/8 diluted cDNA; **lane 5,** 1/16 diluted cDNA; **lane 6,** 1/32 diluted cDNA

## 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 20μl 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-Taq*™ DNA Polymerase per 20μl 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*-StarTaq™ DNA Polymerase | 25161 / 25162 / 25164 |
| *i*-MAX II DNA Polymerase | 25261 / 25263 |
| *i*-pfu DNA Polymerase | 25181 / 25183 |
| *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 |