i-Taq™ DNA Polymerase
QUICK GUIDE

250 units : 25021 | 500 units : 25022

1. KIT DESCRIPTION

iNtRON's i-Taq™ DNA Polymerase is a thermostable DNA polymerase derived from *Thermus aquaticus*. The enzyme exhibits optimal DNA synthesis activity at 72°C and retains sufficient activity during repeated denaturation steps at 94°C, enabling efficient PCR amplification without the need to replenish the enzyme after each cycle. The enzyme is purified to minimize contamination with host-derived DNA, including *E. coli* chromosomal and plasmid DNA. The kit includes 10X PCR buffers, a dNTP mixture, and $MgCl_2$ solution for convenient PCR amplification.

2. CHARACTERISTICS

- **Thermostability :** Maintains enzyme activity during repeated PCR thermal cycling.
- **High Efficiency :** Efficient amplification of target DNA.
- **High Purity :** Purified to minimize contamination with host-derived DNA.
- **Complete Reagent Set :** Includes 10X PCR buffers (with and without $MgCl_2$), dNTP mixture, and 25 mM $MgCl_2$ solution.
- **Versatile Applications :** Suitable for genomic DNA PCR, cDNA amplification, and T/A cloning.

3. KIT CONTENTS AND STORAGE

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

* 10X PCR Buffer : 100 mM Tris-HCl (pH 8.3), 500 mM KCl, 20 mM $MgCl_2$, enhancer solution

- **Storage Condition :** Store at -20°C.

4. CONSIDERATIONS BEFORE USE

- **Research Use Only (RUO) :** For research use only. Not for use in diagnostic or clinical procedures.
- **Storage & Handling :** Store at -20°C. Avoid repeated freeze-thaw cycles.
- **Contamination Control :** Use clean gloves and filter pipette tips to prevent cross-contamination.
- **Template Integrity :** Use high-purity DNA free of inhibitors (phenol, ethanol, EDTA, etc.).

5. PROTOCOLS

1) Prepare the reaction mixture (total 20 µl) as follows :

- **Template DNA** : 1 ng ~ 1 µg
- **Primer 1** : 5 ~ 10 pmoles
- **Primer 2** : 5 ~ 10 pmoles
- **i-Taq™ DNA Polymerase (5 U/µl)** : 0.2 ~ 0.5 µl
- **10X PCR Buffer** : 2 µl
- **dNTP Mixture (2.5 mM each)** : 2 µl
- **Sterile Distilled Water (D.W.)** : up to 20 µl

NOTE : 10X PCR Buffer contains 20 mM $MgCl_2$ (2 mM final). When using 10X $MgCl_2$-free PCR Buffer, add 25 mM $MgCl_2$ to the desired final concentration.

2) Mix gently, spin down briefly, and proceed to PCR.

3) Perform PCR using the following cycling conditions.

[SUGGESTED CYCLING PARAMETERS]

PCR cycle		Temp.	PCR product size 100–500 bp	500–1000 bp	1–5 kb
Initial denaturation		94°C		2 min	
30~40 Cycles	Denaturation	94°C		20 sec	
	Annealing	50–65°C	10 sec	10 sec	20 sec
	Extension	65–72°C	20–30 sec	40–50 sec	1 min/kb
Final extension		72°C	Optional (normally 2–5 min)		

Note : These cycling parameters serve 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 determined individually.

4) Analyze the PCR products by agarose gel electrophoresis.

6. TROUBLESHOOTING GUIDE

Refer to the following troubleshooting guide for possible causes and recommended actions when unexpected PCR results are observed.

Observation	Possible Cause	Recommendation
Little or no PCR product	Primer problems : non-optimal design or concentration, or annealing temperature too high	- Design alternative primers - Reduce annealing temperature - Use 5–20 pmoles of primer per 20 µl reaction - For an established primer pair, check performance with a control template
	Enzyme concentration too low	- Use 1.0–2.5 U of i-Taq™ DNA Polymerase per 20 µl reaction as a starting range. - If necessary, optimize the enzyme amount within 0.1–2.5 U per reaction according to the amplification results.
Multiple bands or background smear	Annealing temperature too low	- Increase annealing temperature in 2°C steps
	Primer design or concentration not optimal	- Review primer design - Titrate primer concentration
	Too high starting concentration of Mg^{2+}, template, cycles, or enzyme	- Reduce one or all of these components
RT-PCR : no product, additional bands, or background smear	Excessive cDNA template	- cDNA (RT reaction) volume should not exceed 10% of the final PCR volume - Titrate cDNA template - Follow the troubleshooting steps above

7. PCR OPTIMIZATION NOTES

- Cycling parameters are provided as general guidelines. Optimal conditions should be determined individually for each target.
- Optimize the annealing temperature (50–65°C) according to the primer Tm.
- Adjust the extension time according to the amplicon length (approximately 1 min/kb for 1–5 kb targets).
- Use 1.0–2.5 U of i-Taq™ DNA Polymerase per 20 µl reaction (0.2–0.5 µl of 5 U/µl enzyme).
- Use 5–20 pmol of each primer per 20 µl reaction.
- For RT-PCR, the volume of the reverse transcription reaction mixture should not exceed 10% of the final PCR reaction volume.

8. CAUTIONS

- Wear appropriate personal protective equipment when handling samples and reagents.
- Use aerosol-resistant filter tips to minimize cross-contamination.
- Use separate areas for PCR setup and post-PCR analysis whenever possible.
- Avoid contamination of reagents with amplified DNA.
- Dispose of biological samples and PCR products according to applicable laboratory procedures.

9. ADDITIONAL REQUIRED EQUIPMENT

- Thermal cycler
- Micropipettes and aerosol-resistant tips
- PCR tubes or plates
- Template DNA and forward/reverse primers
- Sterile distilled water (PCR grade)
- Agarose gel electrophoresis equipment and DNA loading dye

10. EXPERIMENTAL INFORMATION

1) Sensitivity

Serial dilutions of human genomic DNA were amplified with the same amount of i-Taq™ DNA Polymerase. The IL-10 PCR product was detected from as little as 2 pg of genomic DNA.

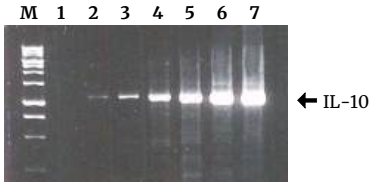


Fig. 1. Total reaction volume 20 µl containing 1 U of i-Taq™. **Lane M,** DNA marker; **1,** negative control; **2–7,** 2 pg, 20 pg, 200 pg, 2 ng, 20 ng, and 200 ng template DNA.

2) Activity per Batch

The β-actin gene (400 bp) was amplified using serially diluted i-Taq™ DNA Polymerase from two production batches (A and B), with human cDNA as the template.

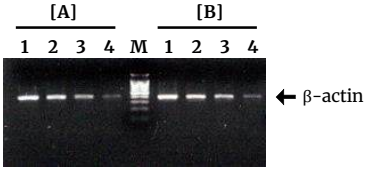


Fig. 2. PCR amplification of the β-actin gene using serial dilutions of i-Taq™ DNA Polymerase from batches A and B. Lane M, DNA marker; lanes 1–4, enzyme dilutions of 2^{-4}, 2^{-5}, 2^{-6}, and 2^{-7}, respectively.

3) PCR amplification

Class 1 integron genes (Panel A) and class 1 integron promoter genes (Panel B) were specifically amplified from *E. coli* genomic DNA (isolated with G-spin™ Genomic DNA Extraction Kit for Bacteria, Cat. No. 17121) using i-Taq™ DNA Polymerase (Cat. No. 25021).

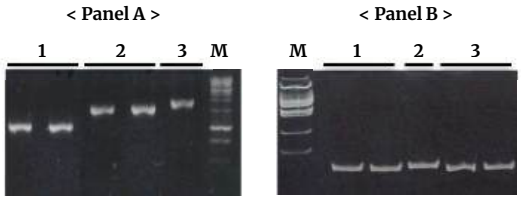


Fig. 3. Panel A : Lane M, 1 kb ladder; **1,** 1.2 kb; **2,** 1.6 kb; **3,** 2.0 kb amplicon. **Panel B : Lane M,** 100 bp ladder; **1, 3,** 80 bp; **2,** 84 bp amplicon.

4) RT-PCR amplification

Total RNA from K562 cells was extracted using the easy-BLUE™ Total RNA Extraction Kit (Cat. No. 17061) and reverse-transcribed using the Power cDNA Synthesis Kit (Cat. No. 25011). Serially diluted cDNA was used to amplify the GAPDH gene with 2.5 U of i-Taq™ DNA Polymerase.

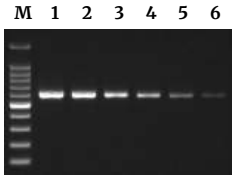


Fig. 4. Lane M, 100 bp ladder; **1,** undiluted cDNA; **2–6,** 1/2, 1/4, 1/8, 1/16, and 1/32 diluted cDNA.

11. EXPLANATION OF SYMBOLS

LOT	Batch code	REF	Catalogue number
Σ	Contains sufficient for <n> tests		Temperature limit
	Date of manufacture		Manufacturer
	Use-by date	RUO	Research Use Only
	Consult instructions for use		Caution

12. RELATED PRODUCTS

Product Name	Cat. No.
i-StarTaq™ DNA Polymerase	25161 / 25162
i-MAX™II DNA Polymerase	25261
i-pfu DNA Polymerase	25181 / 25183
i-StarMAX™ II DNA Polymerase	25173
Maxime™ PCR PreMix (i-Taq)	25025/25026
Maxime™ PCR PreMix (i-StarTaq)	25165/25167
Maxime™ PCR PreMix (i-pfu)	25185
Maxime™ PCR PreMix (i-MAXII)	25265
Maxime™ PCR PreMix (i-StarMAXII)	25281