i-StarTaq™ GH DNA Polymerase

[for antibody-based Hot-Start PCR]

The Best Choice of *i*-StarTaq™ GH DNA Polymerase for Hot-Start PCR.
For standard and specialized end-point antibody-based Hot Start PCR applications without the need for optimization

RUO *Research Use Only* REF 26030 Σ250 REF 26031 Σ500 -18°C -22°C

DESCRIPTION

Due to a possibility of Taq DNA Polymerase activation at 37℃, there may be an extention step of PCR by binding primer with DNA molecular before completing the initial denaturation step. In a case of the annealing step at low temperature, there is a high risk of mismatched primers so it is hard to get clear bands what to see. Anti-Taq antibody is an antibody inhibitor which binds Taq DNA polymerase at low temperature. During PCR reaction, anti-Taq antibody has a specific characteristic not to inhibit activations of Taq DNA Polymerase by coming denatured antibody off in the denaturation step. ***i*-StarTaq ™GH DNA Polymerase** developed with this characteristic has high specificities on target DNA by inhibiting activation at the initial step of PCR. The advantage of ***i*-StarTaq ™GH DNA Polymerase** is to maximize amplified products and simultaneously minimize non-specific reactions. (product) can prevent non-specific priming and oligomerization, so it is easy to get much better PCR products having high yield, sensitivity, specificity and amplification than before.

CHARACTERISTICS

- **Applied Taq Antibody**
- **High Sensitivity :** Reduced or no amplification of non-specific products resulting from mis-priming during PCR.
- **High Specificity :** Hot-start PCR activity shows with a high specificity and accuracy with high amplification yield
- **High reproducibility test result**
- **Flexibility :** Available for various DNA template including cloned fragment, phage DNA, mammalian genomic DNA and etc.
- **Stability :** Stable for over 2 years at -22~-18°C

KIT CONTENTS

Contents	26030(250 Units)	26031(500 Units)
i-StarTaq™ GH DNA Polymerase (5U/㎕)	250 U	500 U
10X PCR Buffer * (w/20 mM Mg^{2+})	1 ml	1 ml
10X Mg^{2+} free PCR Buffer	1 ml	1 ml
10 mM dNTPs (2.5mM/each)	500 ㎕	1 ml
25 mM Mg^{2+}	1 ml	1 ml
Instruction Manual	1 ea	1 ea

* **10X PCR Buffer**- 300 mM Tris-HCl(pH 9.3); 300 mM salts containing of K^+ and NH^{4+}; 20mM Mg^{2+}; Enhancer solution

STORAGE AND STABILITY

- Storage condition : Store the product at -22 ~ 18℃ after receiving.
- Expiration : ***i*-StarTaq™ GH DNA Polymerase** can be stored for up to 2 years without showing any reduction in performance and quality under appropriate storage condition. The expiration date is labeled on the product box.

APPLICATIONS

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

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

At iNtRON we pride ourselves on the quality and availability of our technical support. Our OS center is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If you have any questions or experence any difficulties regarding the ***i*-StarTaq™GH DNA Polymerase**, please do not hesitate to contact us. iNtRON customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at iNtRON. For technical assistance and more information please call iNtRON local distributors.

TECHNICAL ASSISTANCE

***i*-StarTaq™GH DNA Polymerase** is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. ***i*-StarTaq™GH DNA Polymerase** is developed, designed, and sold for research purpose only. It is not intended to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals.

QUALITY CONTROL

In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of ***i*-StarTaq™GH DNA Polymerase** is tested against predetermined specifications to ensure consistent product quality.

Contents	Quality Control
PCR Buffer, dNTP Mixture	Conductivity, pH, sterility, and performance in PCR are tested.
Distilled Water	Conductivity, pH, sterility, and performance in PCR are tested. Endonuclease, exonuclease, and RNase activities are tested.
i-StarTaq™GH DNA Polymerase	PCR reproducibility assay: The PCR reproducibility assay reactions are performed in using 3 batch.
Process Inspection	Accuracy of aliquot process was validated Appearance of Master mix solution (housing, sealing contamination)

ADDITIONAL REQUIRED EQUIPMENT

- Distilled water
- Primers
- Pipettes and pipette tips (aerosol resistant)
- Thermal cycler
- Mineral oil (only if the thermal cycler does not have a heated lid)

IMPORTANT NOTES BEFORE STARTING

- Set up reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- The annealing efficiency of primers to the template is an important factor in PCR. If too little template is used, primers may not be able to find their complementary sequences. Too much template may lead to an increase in mis-priming events.

PROTOCOL

1. Preparation of Reagents

1) ***i*-StarTaq™ GH DNA Polymerase** : Leave it immediately at room temperature before use. Do not leave it at room temperature more than 1 hour.
Note : Be repeated freezing and thawing, the product may have an impact on performance.

2) Template and primer : Sample should be added in primer tube at the separate place.

3) DNase/RNase Free Water : No template Control (NTC)

PROTOCOL (Continued)

2. PCR Protocol

1) **Dispense 2 ㎕ of 10X PCR buffer and 2 ㎕ of dNTP Mixture (in case of total 20 ㎕ PCR reaction) into PCR tubes.**

2) **Add template DNA and primers into upper PCR tubes.**
Note 1: Recommended volume of template: 1 ㎕~2 ㎕
Note 2: Appropriate amounts of DNA template samples
- cDNA : 0.5-10 % of reaction volume
- Plasmid DNA : 10 pg-100 ng
- Genomic DNA : 0.1-1 ㎍

Note 3 : Appropriate amounts of primers
- Primer : 0.1~0.5 μM each (sense and anti-sense)

3) **Add 0.2 ㎕~0.5 ㎕ of *i*-StarTaq™ GH DNA Polymerase (5 U/㎕) into PCR tubes.**

4) **Add distilled water into the tubes to a total volume of 20 ㎕ .**
Note: This example serves as a guideline for PCR amplification. Optimal reaction conditions such as amount of template DNA and amount of primer, may vary and must be individually determined.

Example

PCR reaction mixture	20 ㎕ rxn Add
Template DNA (1 ng-1 ㎍)	1~2 ㎕
Primer (F : 10 pmol/㎕)	1 ㎕
Primer (R : 10 pmol/㎕)	1 ㎕
i-StarTaq™ GH DNA Polymerase (5 U/㎕)	0.2~0.5 ㎕
10X PCR buffer	2 ㎕
dNTP Mixture (2.5 mM each)	2 ㎕
Sterilized distilled water	Up to 20 ㎕

5) **Mix the mixture well by pipetting or vortexing then spin down the mixture by brief centrifugation.**

6) **(Option) Add mineral oil.**
Note: This step is unnecessary when using a thermal cycler that employs a top heating (general methods) .

7) **Perform PCR of samples.**

Suggested cycling parameters

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

Note : (1) Depending on the specific TM Primers
(2) 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.

8) **Load samples on agarose gel adding a loading-dye buffer and perform electrophoresis.**
Note: RedSafe ™ Nucleic Acid Staning Solution (iNtRON, Cat. No. 21141) and Agarose L E (iNtRON, Cat. No. 32034) are recommended.

EXPERIMENT INFORMATIONS

❖ Performance of *i*-StarTaq™ GH DNA Polymerase in PCR

i-StarTaq™ GH DNA Polymerase provides the detection of PCR band with high sensitivity and amplification yield than *i*-StarTaq™ DNA Polymerase.

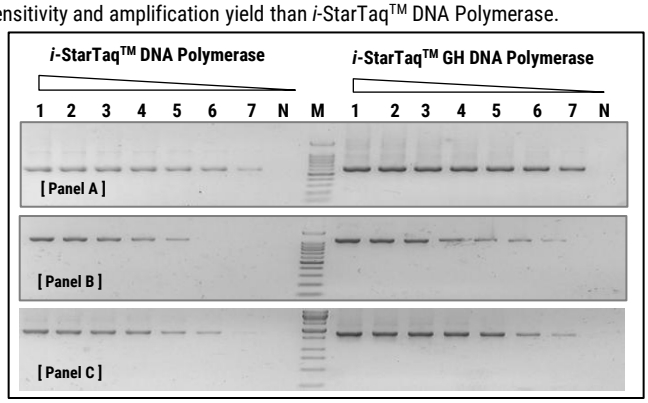

Fig 1. PCR amplification of various PCR Results with i-StarTaq™ GH DNA Polymerase and i-StarTaq™ DNA Polymerase
[Panel A] GAPDH (570 bp); **[Panel B]** 1.3 Kb amplification; **[Panel C]** 1.8 Kb amplification
[Panel A/B/C]
Lane M, SiZer™-100 plus DNA Marker Solution(Cat. No. 24072); **lane N,** Negative Control
Lane 1, 100 ng K-562 gDNA; **lane 2,** 10 ng K-562 gDNA; **lane 3,** 1 ng K-562 gDNA; **lane 4,** 100 pg K-562 gDNA; **lane 5,** 10 pg K-562 gDNA; **lane 6,** 1 pg K-562 gDNA; **lane 7,** 100 fg K-562 gDNA

❖ Performance of *i*-StarTaq™GH DNA Polymerase in Molecular diagnosis PCR

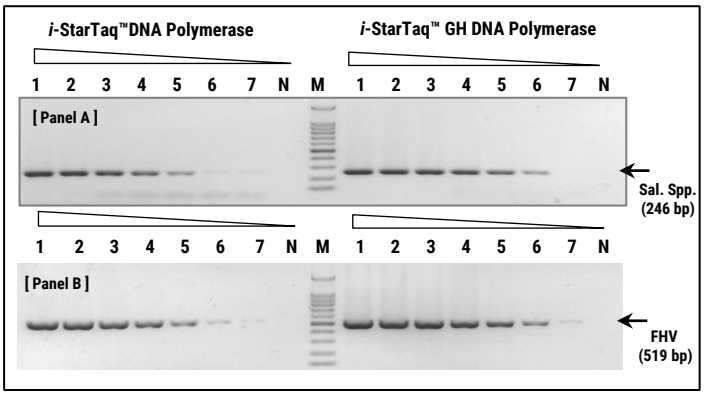

Fig 2. PCR amplification of Molecular diagnosis PCR Results with i-StarTaq™ GH DNA Polymerase and i-StarTaq™ DNA Polymerase
[Panel A] *Salmonella spp.* Detection; **[Panel B]** FHV Detection
[Panel A], Lane M, SiZer™-100 plus DNA Marker Solution(Cat. No. 24072); **lane 1 ~ 7,** 1/10 serial diluted *Salmonella spp.* DNA; **lane N,** Negative control
[Panel B], Lane M, SiZer™-100 plus DNA Marker Solution(Cat. No. 24072); **lane 1 ~ 7,** 1/10 serial diluted FHV DNA; **lane N,** Negative control

TROUBLESHOOTING GUIDE

Symptoms & Possible Causes	Comments & Suggestions
Little or no product	
1) Pipetting error or missing reagent	• Repeat the PCR. Check the concentrations and storage conditions of the kit, primers and template.
2) Primer concentration is not optimal or primers degraded	• Repeat the PCR with different primer concentrations from 0.1–0.5 μM of each primer (in 0.1 μM increments).
3) Problems with starting template	• Check the concentration, storage conditions, and quality of the starting template. If necessary, make new serial dilutions of template nucleic acid from stock solutions. Repeat the PCR using the new dilutions
4) Insufficient number of cycles	• Increase the number of cycles in increments of 5 cycles.
5) Hot Start function is not activated	• Check whether PCR was started with an initial denaturation Step at 95℃ for 5 min.
6) Incorrect annealing temperature or time	• Decrease annealing temperature by 2℃ increments.
7) Incorrect denaturation temperature or time	• Annealing time should be between 1 - 2 m Adjust the time in increments of 5 s.
8) Extension time too short	• Increase the extension time by increments of 30 s.
9) Primer design is not optimal	• Review primer design.
10) cDNA concentration	• For RT-PCR, take into consideration the efficiency of reverse transcriptase reaction which averages 10–30%. As RT reaction Mix is known to be a PCR inhibitor. The added volume of the cDNA should not exceed 10% of the final PCR volume.
11) Cycle number is too low	• Repeat the PCR. Check the concentrations and storage.
12) Template with a high degree of secondary structure e conditions of the kit, primers and template.	• Increase the cycle number in increments of three cycles.

RELATED PRODUCTS

Product Name	Cat. No.
i-Taq™ DNA Polymerase	25021 / 25022
i-StarTaq™ DNA Polymerase	25161 / 25162
i-MAX II DNA Polymerase	25261
i-StarMAX II™ DNA Polymerase	25173
i-pfu DNA Polymerase	25181
RealMOD ™Real-time PCR Master mix Kit (2X)	25341 / 25342
RealMOD™ Green Real-time PCR Master mix Kit (2X)	25343 / 25344