Instruction manual for Hot Start rTth (DNA) Kit 2103

F1768K

Hot Start rTth (DNA) Kit

HSTTH-301 250U

Store at -20°C

Contents

CAUTION

All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe general laboratory precautions and safety while using this kit.

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[1] Introduction

Description

Hot Start rTth (DNA) Kit is PCR reagent based on Tth DNA Polymerase. Tth DNA Polymerase has higher amplification efficiency than Taq DNA Polymerase, which is a general-purpose enzyme, and enables amplification from low copies of template and amplification from a crude sample containing PCR inhibitors.

In addition, Tth DNA Polymerase has a 5 '- 3' exonuclease activity, so it can be used for real-time PCR using probe assays such as TaqMan® assay. This enzyme contains neutralizing antibodies, thus allowing for Hot start PCR.

Features

-Excellent DNA Amplification Efficiency

The reaction composition is optimized based on Tth DNA Polymerase, enabling efficient PCR even from low copies of template.

- Tolerant of PCR Inhibitors

This kit is effective for amplification from crude samples (*e.g.*, biological samples, foodstuffs, soil extract, etc.). In the case of amplification from whole blood, sufficient amplification can be achieved by adding it directly to the reaction solution without purification of nucleic acid.

- Utilization of dUTP

This kit contains dUTP instead of dTTP in 2x Buffer for rTth/ TTx (DNA). Therefore, the rate of false-positive detection can be reduced by adding uracil-N-glycosylase (UNG).

*UNG is not supplied with this kit.

[2] Components

This kit includes the following components for 250 reactions, 20 µL total reaction volume. All reagents should be stored at -20°C.

2x Buffer for rTth/ TTx (DNA)	1.25 mLx 2
Hot Start rTth DNA Polymerase (4U/ µL)	62.5 µL

Note:

-2× Reaction Buffer contains essential components for the reaction (buffer, salts, Mg^{2+}, dATP, dCTP, dGTP, and dUTP, etc.). Add template DNA, primers, and attached Hot Start rTth DNA Polymerase, and adjust to 1x concentration with sterile water etc.

-DNA Polymerase is a mixture of rTth DNA polymerase and hot start antibodies. Its concentration is 4U/ µL.

-This kit doesn't contain a passive reference dye (ROX). When using a passive reference dye to compensate fluorescence intensity and dispensing error between wells, please use the separately sold 50x ROX reference dye (Code No. ROX-101).

[3] Protocol

1. Standard reaction setup

Before preparing the mixture, all components should be completely thawed, except for the enzyme solution.

Components	Volume	Final Concentration
PCR grade water	X µL	
2x Buffer for rTth/ TTx (DNA)	10 µL	1x
10 µM Primer #1	0.6 µL	0.3 µM
10 µM Primer #2	0.6 µL	0.3 µM
TaqMan® Probe(10 µM)	0.4 µL	0.2 µM
Hot Start rTth DNA Polymerase	0.25 µL	1U
Template DNA (Sample)	Y µL	
Total reaction volume	20 µL	

Notes:

-The recommended amount of primer should be 0.2-0.6 µM, and the amount of TaqMan® probe should be 0.05-0.3 µM. If amplification efficiency is not good, performance may be improved by increasing the addition amount, but if it is added too much, it may cause non-specific reaction and detection sensitivity may be lowered.

2. Cycling conditions

The following cycle is recommended.

	Temperature	Time	
Predenature :	95°C	1 min.	
Denature :	95°C	15 sec.	] 40~50 cycles
Annealing/extension :	60°C	30 sec.	

Notes:

[Optional] The uracil-N-glycosylase (UNG) treatment step should be added before predenature step. The indicated temperature and time are typical conditions for UNG. The conditions can be optimized according to the particular instruction manual from the supplier of UNG.

- If sensitivity is not good, it may be improved by changing annealing/ extension temperature between 55 ~ 65°C.

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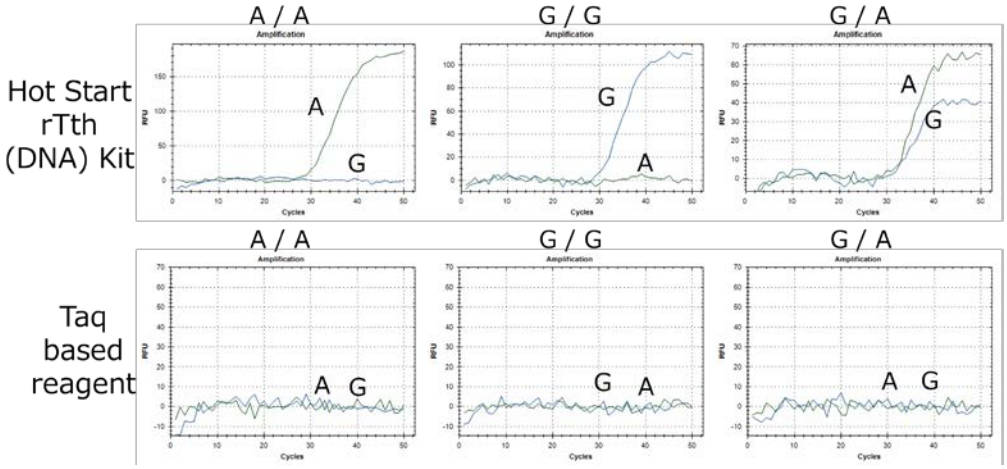
[4] Example

Detection of SNPs

SNPs in aldehyde dehydrogenase 2 (ALDH2) gene were detected using TaqMan® probes. 1 µLof whole blood was added to 20 µLreaction mixture as template. As a result, Taq DNA Polymerase-based reagent was inhibited by whole blood and amplification could not be confirmed. On the other hand, Hot Start rTth (DNA) Kit was able to determine SNPs from whole blood without purification.



[5] Related products

Product name	Package	Code No.
<High efficient DNA Polymerase for PCR and RT-PCR> **Hot Start rTth DNA Polymerase**	10,000 U	HSTTH-329
<Reaction Buffer (containg Mg^{2+}) for DNA amplification> **2x Buffer for rTth/ TTx (DNA)**	100 mL 250 mL 1,000 mL	QRZ-1B1 QRZ-1B2 QRZ-1B4
<Passive reference dye> **50x ROX reference dye**	5 mL	ROX-101

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