F1788K

Hot Start TTx (RNA) Kit

HSTTX-111 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 TTx (RNA) Kit is RT-PCR reagent based on our original polymerase, TTx DNA Polymerase. TTx DNA polymerase exhibits reverse transcriptase activity in the presence of Mn2+ ions. This system allows for 1-step real-time PCR, including reverse transcription and PCR steps.

TTx DNA Polymerase has higher amplification efficiency than Taq DNA Polymerase, which is a general-purpose enzyme, and has reverse transcription activity, enabling amplification from a crude sample containing PCR inhibitors with high efficiency from both DNA and RNA.

In addition, TTx 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

- Enables highly efficient 1-enzyme 1-step RT-PCR

TTx DNA Polymerase has high reverse transcription activity and enables amplification from low copies of template and enable efficient PCR even fast cycle condition.

In addition, TTx DNA Polymerase is a thermostable enzyme and can reverse transcribe at high temperatures above 60°C. It is suitable for amplifying GC rich targets and targets that form higher order structures.

This Kit can also be applied to DNA amplification.

[2] Components

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

5x Buffer for rTth/ TTx (DNA/ RNA)	1 mL
2 mM dNTPs	1 mL
50 mM $Mn(OAc)_2$	250 µL
Hot Start TTx DNA Polymerase (4U/ µL)	62.5 µL

Note:

-5x Reaction Buffer for rTth/ TTx (DNA/ RNA) is 5x RT-PCR buffer not containing Mn^{2+} and dNTPs. Add template DNA, primers, and attached 2 mM dNTPs, 50 mM $Mn(OAc)_2$, Hot Start TTx DNA Polymerase, and adjust to 1x concentration with sterile water etc.

-DNA Polymerase is a mixture of TTx 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	
5x Buffer for rTth/ TTx (DNA/ RNA)	4 µL	1x
2 mM dNTPs	4 µL	0.4 mM
50 mM $Mn(OAc)_2$	1 µL	2.5 mM
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 TTx DNA Polymerase	0.25 µL	1U
Template RNA or 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 1 :	90°C	30 sec.	
Reverse Transcription:	60°C	5 min.	
Predenature 2:	95°C	1 min.	
Denature :	95°C	15 sec.	] 40~50 cycles
Annealing/extension :	60°C	30 sec.	

Notes:

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

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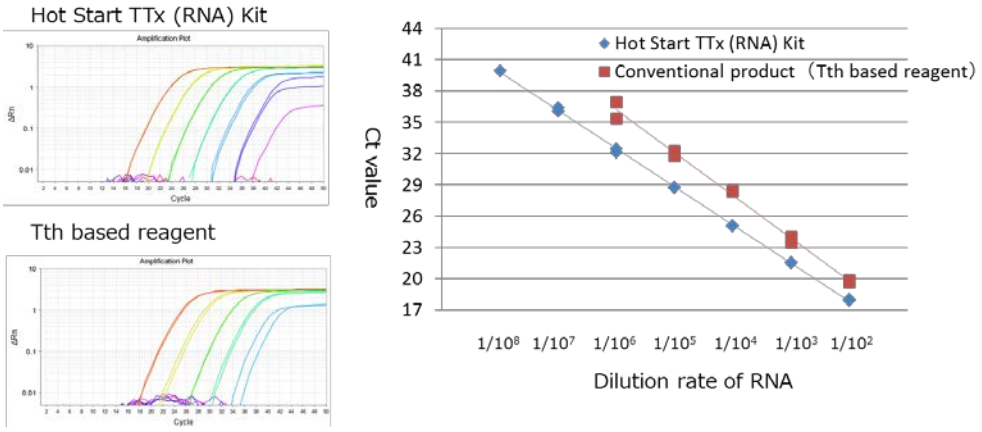
[4] Example

Detection of influenza virus

Influenza RNA was detected using TaqMan® probes. As a result of adding 1 µL of diluted purified RNA to 20 µL of the reaction solution, Hot Start TTx (RNA) Kit obtained higher sensitivity than Tth DNA Polymerase-based reagent. By using TTx DNA Polymerase, efficient detection is possible even from low copies of template.



[5] Related products

Product name	Package	Code No.
<High efficient DNA Polymerase for PCR and RT-PCR> **Hot Start TTx DNA Polymerase**	10,000 U 100,000 U	HSTTX-129 HSTTX-159
<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
<High efficient DNA Polymerase for PCR and RT-PCR> **Hot Start rTth DNA Polymerase**	10,000 U	HSTTH-329
< Reaction Buffer (not containg Mg^{2+} and Mn^{2+}> **5x Buffer for rTth/ Ttx (DNA/ RNA)**	40 mL 400 mL	QRT-1B1 QRT-1B2
<Mn solution for RNA amplification> **50 mM $Mn(OAc)_2$**	5 mL	QRT-MN1
<Mg solution for DNA amplification> **25 mM $MgCl_2$**	40 mL	TAP-2S1

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