UltraBio™ Hotstart HiTaq HS III One-step RT-PCR Mix

FP1509100

Storage -20℃. Avoid freeze/thaw cycle.
Shipping Ultra-low temperature transportation.

Introduction

Enzyme System (Hotstart HiTaq & Script III), renowned for its exceptional speed and quantitative performance, is widely used. Real Time RT-PCR reactions using this product can be performed consecutively within the same reaction tube, offering simple operation and effective contamination prevention. This product incorporates a reverse transcriptase with higher thermal stability (HS Script III RTase) and a chemically modified hot-start enzyme (Hotstart HiTaq DNA Polymerase), providing high amplification efficiency and specificity. It enables stable Real Time One-Step RT-PCR reactions, significantly improves detection sensitivity, and eliminates the need for post-PCR electrophoresis. It is particularly suitable for detecting low-abundance RNA, such as from RNA viruses.

This product is specifically designed for convenient and highly sensitive One-Step RT-PCR. Its unique enzyme system and specially formulated buffer ensure efficient and accurate reverse transcription and PCR reactions without the need for optimization. It yields excellent standard curves over a wide quantitative range, allowing for accurate quantitative detection of target genes with good repeatability and high reliability.

Scope of Application

Suitable for One-Step amplification of RNA or hot-start DNA amplification.

Component List

FP1509100	Component	100T	200T	Storage
FP1509100A	4× RT PCR Buffer	625 μL	1.25 mL	-20℃.
FP1509100B	Hotstart HiTaq& Script III	100 μL	200 μL	-20℃.

Note: The number of reactions corresponding to the catalog number is calculated based on a 25 μL reaction volume.

Instructions for Use

1. Preparation of Reaction Mixture

1.1. Thaw and mix all solutions required for the PCR reaction. After a brief centrifugation, place them at room temperature for use. Probes should be protected from light. It is recommended to aliquot PCR reagents to avoid repeated freeze-thaw cycles.

1.2. Prepare the PCR reaction mixture according to the table below:

Component	Volume for 25 μL System	Volume for 50 μL System
4× RT PCR Buffer	6.25 μL	12.5 μL
Enzyme System (Hotstart HiTaq&Script III)	1 μL	2 μL
Primer-probe Mix	1 μL ※	2 μL ※
Templates	5 μL ※	10 μL ※
ddH_2O	Up to 25 μL	Up to 50 μL
Total	25 μL	50 μL

※ Note: The amounts of Primer-probe Mix and Templates can be adjusted as needed.

1.3. Mix gently by pipetting or by brief vortexing. Centrifuge briefly at room temperature to collect all liquid at the bottom of the tube.

1.4. Place the PCR reaction tubes on a pre-programmed thermal cycler and start the PCR reaction.

2. Reaction Protocol

Step	Temperature	Time	Cycles
1	50°C	15 min	1
2	95°C	10 min	1
3	**95°C**	**10 sec**	**45 (2)**
4	**55°C (1)**	**40 sec (Fluorescence Acquisition)**	

3. Notes:

a. The typical final concentration for each primer is 0.2 μM. The Tm value of the primers is generally recommended to be set above 55°C. Analysis with relevant software is advised. Using optimal primer concentrations and annealing temperatures will yield ideal reaction results.

b. For most experiments, 45 cycles are sufficient. If your samples have a high target content, the cycle number can be appropriately reduced (e.g., to 40 cycles) to shorten the detection time.