One Step RT-PCR Kit (cDNA synthesis + PCR in one reaction)

Catalog number: MB1003

Contains all reagents needed for first-strand cDNA synthesis except the templates. Downstream applications include RT-PCR.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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antibody and ELISA experts

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One Step RT-PCR Kit (cDNA synthesis + PCR)

Catalog Number:MB1003

Assay Principle

Boster's One Step RT-PCR Kit contains all the reagents necessary for reverse transcription and PCR amplification to occur in a single PCR reaction tube, without the template. The kit contains an optimal recombinant Reverse Transcriptase and HotStart h-Taq DNA Polymerase mixture. This provides dual advantages of high efficiency for cDNA synthesis and high specificity of hot start polymerase, to set up optimal PCR conditions easily and conveniently. This product offers the user an efficient and reliable alternative to conventional “two-step” RT-PCR. Gene-specific primers must be used along with this kit.

Overview

Product Name	One Step RT-PCR Kit (cDNA synthesis + PCR in one reaction)
SKU/Catalog Number	MB1003
Description	The One Step RT PCR Kit provides an efficient, accurate, and convenient way to synthesize cDNA and amplify via PCR, from any template, compatible with both Poly(A) mRNA and total RNA. This kit saves time and reduces contamination due to a reduced number of pipetting steps required for RT PCR set up.
Cite This Product	One Step RT-PCR Kit (cDNA synthesis + PCR in one reaction) (Boster Biological Technology, Pleasanton CA, USA, Catalog # MB1003)
Application	RT-PCR *Our Boster Guarantee covers the use of this product in the above tested applications.
Pack Size	100 Reactions
Shelf life	1 year (for storage at -20°C).

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List of Components

Components	Size
One Step RT-PCR Enzyme Mix	0.2 ml
5X One Step RT-PCR Buffer	1 ml
10 mM dNTP Mix (dATP, dTTP, dGTP, dCTP)	0.2 ml
5X Band Sharpener	1 ml
RNase-free Water	1 ml

Assay Protocol

RT-PCR should be assembled in a nuclease-free environment. RNA sample preparation, reaction mixture assembly, PCR and subsequent reaction analysis should be performed in separate areas. The use of “clean”, automatic pipettors designated for PCR and aerosol resistant barrier tips are recommended.

1. Thaw RNA templates and the MasterMix on ice.
2. Prepare the following reaction mixture in a PCR tube on ice:

Components	Reaction volume: 30ul
5X One Step RT-PCR Buffer	6 ul
10mM dNTP mix	1 ul
Forward Primer (10 pmole /µl)	1 ul
Reverse Primer (10 pmole /µl)	1 ul
Template RNA	- ul
5X Band Sharpener	0-6ul
One Step RT-PCR Enzyme Mix	2 ul
Add RNase-free water (provided) to	30 ul

Notes:
1. Gene specific primers must be used.
2. Template should be <300 ng.
3. Gently mix and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.
4. You may modify the amount of template, extension time, annealing temperature, and the number of PCR cycle according to the target size, primer’s Tm, and the type of templates for amplification.
5. Program the thermal cycler as follows:

Step	Temperature	Duration	Cycles
cDNA Synthesis	50°C	30 min	1
Pre-Denaturation	95°C	15 min	1
Denaturation	95°C	20 sec	35-40
Annealing	AT	40 sec	35-40
Extension	72°C	1 min/kb	35-40
Extension	72°C	5 min	1
Completion	8°C	∞	

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Example of using with 5X Band Sharpener (Reaction vol. 30 ul):

Improved results can be obtained by adjusting of 5X Band Sharpener if template has high G+C region or secondary structure. Please add 5X Band Sharpener to a final concentration of 0.5X-2X (5-20ul for 50ul reaction) if needed.

Reaction Mix	Mix I (0x)	Mix II (0.5x)	Mix III (1x)
5X One Step RT-PCR Buffer	6 ul	6 ul	6 ul
10 mM dNTP mix	1 ul	1 ul	1 ul
Forward Primer (10 pmole/ul)	1 ul	1 ul	1 ul
Reverse Primer (10 pmole/ul)	1 ul	1 ul	1 ul
Template RNA	- ul	- ul	- ul
5X Band Sharpener	0 ul	3 ul	6 ul
One Step RT-PCR Enzyme Mix	2 ul	2 ul	2 ul
Add Rnase-free water (Provided) to	30 ul	30 ul	30 ul

General Notes

1. Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
2. RNA samples must be free of genomic DNA contamination.
3. Components are light sensitive; avoid prolonged exposure to intense light.
4. Start reaction as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to RT-PCR reaction.