

One-Step Sybr Green qRT-PCR Kit

DESCRIPTION

Tribo™ One-Step Sybr Green qRT-PCR Kit is a complete qRT-PCR system containing all necessary reagents for both reverse transcription and PCR amplification to occur in a single qPCR reaction tube. Specifically, this One-Step qRT-PCR kit contains a qRT-PCR Enzyme Mix and a Sybr Green qPCR Master Mix. Our proprietary qRT-PCR Enzyme Mix contains stabilizers and enhancers to optimize the two reactions in a real-time “single step”. This One-Step qRT-PCR kit offers the end-users an efficient, easy to use and reliable alternative to conventional “two-step” sequential qRT-PCR. Gene-specific primers must be used along with this kit.

APPLICATIONS

This kit is used for real-time PCR amplification.

KEY FEATURES

- ❖ One-step complete qRT-PCR in a single tube.
- ❖ Reduce contamination in operating process.
- ❖ Accurate detection and quantification of target gene through real time PCR.

KIT CONTENTS

Part Number	Component	Kit Catalog Number		
		TBS4007R	TBS4007iC	TBS4007S
qRT-PCR MixR	SyBr Green qPCR ROX	1.25 ml		-
qRT-PCR MixiC	Sybr Green qPCR iCycler		1.25 ml	
qRT-PCR MixS	Sybr Green qPCR-No Dye		-	1.25 ml
RT003	qRT-PCR Enzyme Mix (50X)	40 µl		
RT-0	Nuclease-free H ₂ O	1 ml		
Size		100x 20µL Reactions		

STORAGE CONDITIONS

Store all components at -20°C in a non-frost-free freezer. Shelf life is 12 months after recipient. The kit is shipped on ice.

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. Prepare the following reaction mixture in PCR tube on ice

Components	Reaction Volume: 20 µl	Concentration
Total RNA	Variable	2 pg - 0.2 µg/rxn
Bybr qRT-PCR Mix	10 µl	1X
qRT-PCR Enzyme Mix (50X)	0.4 µl	1X
Forward Primer (6 µM)	1 µl	300 nM
Reverse Primer (6 µM)	1 µl	300 nM
Nuclease-free H O	Up to 20 µl	

Note:

1. Gene specific primers must be used.
 2. Amplicon should be <150 bp in size.
 3. Please ensure no salt crystals are present in the Sybr Green qPCR Master Mix before use. If salt crystals are observed, mix until crystals are completely dissolved and absent.
2. Gently mix and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.
 3. Program the thermal cycler so that cDNA synthesis is followed immediately by qPCR amplification.

Step	Temperature	Duration	Cycle(s)
cDNA Synthesis	42°C	15 mins	1
Pre-Denaturation	95°C	10 mins	1
Denaturation	95°C	15 secs	40
Annealing	60°C	60 secs	
Melt Curve	According to the instrument guidelines		

Recommendations for Optimal Results

- Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
- Sybr Green qPCR MasterMix components are light sensitive; avoid prolonged exposure to intense light.
- Start reaction as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to qRT-PCR reaction.

RELATED PRODUCTS

One-step Taqman Probe qRT-PCR (catalog# TBS4008)
 2xSybr Green qPCR Mix (catalog# TBS4001)
 2xTaqman Probe qPCR Mix (catalog# TBS4002)
 RNA isolation Kit (TBS6001)
 Ribospine vRD II Kit (Viral RNA isolation from cell-free fluid, plasma, Serum,Urine) (catalog# 322-150)
 Exgene Viral DNA/RNA Isolation Kit (Cata#128-150)

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