molecular designs

PRODUCT INFORMATION

Catalog No.: G493

Product Name: **One-Step TaqProbe qRT-PCR Kit**

Size: 100 rxns

DESCRIPTION: **One-Step TaqProbe qRT-PCR Kit** is a complete quantitative RT-PCR kit for TaqMan assay in one step. It contains all reagents necessary for both Reverse Transcription (RT) and TaqMan Probe based qPCR amplification to occur in a single qPCR reaction tube. The **One-Step TaqProbe qRT-PCR Kit** kit is an integration of two key processes: the RT and the real-time PCR. With our innovative RT Master Mix, stabilizers and enhancers, and the hot-start qPCR enzyme, it enables a seamless coupling of two separate steps into one real-time "single step" process. It is very easy and useful for gene expression study and other practical applications.

Kit Contents:	qPCR TaqProbe MasterMix, 2X	qRT-PCR Enzyme Mix	ROX dye Solution	Nuclease-free H_2O
	1.25 ml	40 µl	15 µl	1 ml

Storage: Stored at -20°C

GENERAL PROTOCOL:

Note: Before start, determine the needed ROX dye solution: 1). No ROX: use the kit directly. 2). Low ROX: add 1µl of the ROX dye solution to the 1.25 ml qPCR MasterMix-R tube and mix. 3). High ROX: add 11 µl of the ROX dye solution to the 1.25 ml qPCR MasterMix-R tube and mix.

GENERAL PROTOCOL:

1. Prepare the following reaction in a qPCR tube or plate:

Components	Volume	Final Concentration
Total RNA, or mRNA	X µl	Total RNA: 2pg-0.2 µg/rxn, or mRNA: 0.01 pg -2 ng/rxn
TaqProbe qPCR MasterMix	10 µl	1X
qRT-PCR Enzyme Mix, 50X	0.4 µl	1X
Forward and reverse primers	2 µl	Variable (100-600nM)
TaqMan Probe	1 µl	Variable (100-300nM)
Nuclease-free H_2O	Y µl	--
Total final volume	**20 µl**	**--**

2. Gently mix and make sure the contents are all at the bottom of the tube. Centrifuge briefly in needed.
3. Program the qPCR machine using the following cycling program:

Step	Temperature	Duration – Standard	Cycle(s)
cDNA Synthesis	50°C	15 min	1
Pre-Denaturing	95°C	3 min	1
Denaturing	95°C	15 sec	40
Annealing/extension	60°C	60sec	
Melting Curve	According to the instrument guidelines		

Recommendations for Optimal Results

- For transcripts >9kb, yield can be increased by incubating the cDNA synthesis step to 30-50 min.
- TaqProbe qPCR MasterMix components are light sensitive; avoid exposure to light.
- Start PCR as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to PCR reactions.