EzWay™ One-Step RT-PCR Universal qMasterMix

1. Catalog No.

Cat. No	Product	Size
K0569520	EzWay™ One-Step RT-PCR Universal qMasterMix	1.0ml

2. Storage

1 year at -20°C
(The product is able to be shipped on blue ice and should be stored immediately at -20°C.)

3. Contents

Component	Cat. No	Packing	Comments
2X EzWay™ One-Step RT-PCR Universal qMasterMix	K0569520	1.0ml	Mixture of Hot Taq DNA Polymerase, qPCR Buffer, dNTP, MMLV Reverse Transcriptase and additives
4X Magic Buffer	K0561031	1.0ml	**Only use for High GC content**

4. Description

EzWay™ One-Step RT-PCR Universal qMasterMix is designed for sensitive and specific amplification one-tube reaction from total RNA transcripts. And this kit is used the 5'-nuclease process for the quantitative RT-PCR, such as TaqMan assay.
EzWay™ One-Step RT-PCR Universal qMasterMix is a premixed buffer solution containing MMLV Reverse Transcriptase and Hot Taq DNA Polymerase, dNTPs and stabilizers. The solution contains the proper concentration of $MgCl_2$ and dNTPs for the better specificity and sensitivity and supplied as 2X concentrated type for convenience.
Simply add primers and RNA template diluted in DEPC-treated water into equal volume of EzWay™ One-Step RT-PCR Universal qMasterMix.
Pipetting steps are minimized, reducing the possibility of errors and contamination.
This solution does not contain ROX reference dye, which allows fluorescence normalization on cyclers, such as ABI7000, ABI7300, ABI7700, ABI7900HT, ABI7500, Stratagene Mx3000, Mx3005P, and MX4000.

- Synthesis of cDNA and sequentially PCR amplification from target gene in one-tube
- Real-time quantitative PCR of cDNA targets using fluorescent probe.
- Specialized buffer composition for highly sensitive and specific RNA amplification
- Simple set up and minimal optimization

5. Application

- Rapid detection and quantification of target gene transcript from total RNA or purified mRNA

6. PCR Amplification

This protocol serves as a guideline for one-step RT-PCR. Reverse transcription and PCR are carried out sequentially in the same tube. Optimal reaction conditions, such as incubation times, temperatures, and amount of template RNA, may vary and must be individually determined.

Important notes before starting

- EzWay™ One-Step RT-PCR Universal qMasterMix requires initial activation by incubation at 95°C for 15 min before amplification can take place. This incubation also inactivates the reverse transcriptases. Do not heat to activate Hot Taq DNA Polymerase until the reverse transcriptase reaction is finished.
- EzWay™ One-Step RT-PCR Universal qMasterMix is designed to be used with gene-specific primers at a final concentration of 0.2-0.6 µM. The use of random oligomers or oligo-dT primers is not recommended since it will result in the amplification of nonspecific products.
- Set up all reactions on ice.
- Make sure the thermal cycler is preheated to 42-55°C before placing samples in it.
- EzWay™ One-Step RT-PCR Universal qMasterMix contains the proper concentration of $MgCl_2$ which will produce satisfactory results in most cases. However, if a higher Mg^{2+} concentration is required, increase $MgCl_2$ conc. with the higher conc. of $MgCl_2$ solution.
- RNase-free environment should be maintained during RNA isolation and reaction setup.
- Set up the reaction mixtures in an area separate from that used for RNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

1. **Thaw primer solutions**
   Keep on ice after complete thawing, and mix well before use.
   Optional: Prepare a primer mix of an appropriate concentration (see below) using the RNase-free water. This is recommended if several amplification reactions using the same primer pair are to be performed. The final volume of diluted primer mix plus the template RNA, added at step 4, should be 25 µl per reaction.

Component	Final Concentration	Volume/reaction
2X EzWay™ One-Step RT-PCR Universal qMasterMix	1X	25 µL
5’ Primer	0.4 uM	Variable
3’ Primer	0.4 uM	Variable
Probe	0.1-0.2 uM	Variable
Distilled water	-	Variable
Template RNA	total RNA : 1 ng ~ 2 µg Poly(A)+ RNA : 10 pg ~ 500 ng	Variable
Total reaction volume		**50 µL**

2. **Mix by vortexing briefly, and dispense 25 µl into each PCR tube according to Table 1.**
   It is important to mix EzWay™ One-Step RT-PCR Universal qMasterMix before use to avoid localized differences in salt concentration. EzWay™ One-Step RT-PCR Universal qMasterMix is provided as a 2X concentrate (i.e., a 25-µl volume of the EzWay™ One-Step RT-PCR Universal qMasterMix is required for amplification reactions with a final volume of 50 µl). For volumes smaller than 50 µl, the 1/1 ratio of EzWay™ One-Step RT-PCR Universal qMasterMix to diluted primer mix and template should be maintained as defined in Table 1. A negative control (without template RNA) should be included in every experiment. It is recommended that the PCR tubes are kept on ice until they are placed in the thermal cycler.

3. **Distribute the appropriate volume of diluted primer mix into the PCR tubes containing EzWay™ One-Step RT-PCR Universal qMasterMix.**

4. **Add template RNA to the individual PCR tubes.**

5. **Program the thermal cycler according to the program outlined in the table below either A or B.**

Method A

Step		Temp.	Time	Cycles
Reverse Transcription		42-55°C	30-60 min	1
Initial Denaturation		95°C	15 min	1
Cycling	Denaturation	94°C	15 sec	35-45
	Annealing	50-60°C	30 sec	
	Extension	72°C	30 sec	
Final extension		72°C	10 min	1

Method B

Step		Temp.	Time	Cycles
Reverse Transcription		42-55°C	30-60 min	1
Initial Denaturation		95°C	15 min	1
Cycling	Denaturation	94℃	15 sec	35-45
	Annealing & Extension	50-60℃	45-90 sec	
Final extension		72°C	10 min	1

Note: Pre-heating is required to make DNA polymerase active. Reverse transcriptase is inactivated and the cDNA template is denatured.

6. **Start the RT-PCR program while PCR tubes are still on ice. Wait until the thermal cycler has reached 50°C. Then place the PCR tubes in the thermal cycler.**
   Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer-term storage.