EzWay™ Multiplex PCR qMasterMix

1. Catalog No.

Cat. No	Product	Size
K0567820	EzWay™ Multiplex PCR qMasterMix (2X)	1ml
K0567830	EzWay™ Multiplex PCR qMasterMix (2X) with ROX	1ml

2. Storage

1 year at -20°C (Stable for 1 month at 4°C)
(The product is able to be shipped on blue ice and should be stored immediately at -20°C.)
Avoid repeated freeze-thawing.

3. Contents

Component	Cat.No	Comment
2X EzWay™ Multiplex PCR qMasterMix	K0567820	Mixture of Hot Taq DNA Polymerase, dNTP, Reaction buffer, dNTP and additives
	K0567830	K0567830 contains ROX.
4X Magic Buffer	K0561031	For High GC content

4. Description

EzWay™ Multiplex PCR qMasterMix is a premixed solution for multiplex real-time PCR quantification of DNA and cDNA targets (up to 5 targets). It contains Hot Taq DNA Polymerase, the specialized reaction buffer for probe-based Multiplex qPCR, and dNTPs.
Hot Taq DNA Polymerase, a chemically modified form of Taq DNA Polymerase, sustains an inactive form at ambient temperature and activated by a 15 minutes at 95°C. Thus it provides high specificity in hot-start PCR. Also, the enzyme has 5'-nuclease activity required for some real time qPCR, e.g. TaqMan assay.
EzWay™ Multiplex PCR qMasterMix guarantees the highly specific and reproducible quantitative results.
EzWay™ Multiplex PCR qMasterMix with ROX is supplemented with the proper ROX [passive reference dye] concentration to allow fluorescence normalization on cyclers, such as ABI7000, ABI7300, ABI7700, ABI7900HT, ABI7500, Stratagene Mx3000, Mx3005P, and MX4000.

- Multiplex real-time PCR
- Real-time quantitative PCR of DNA and cDNA targets using a fluorescent probe
- High PCR specificity and sensitivity
- Simple reaction setup at room temperature
- Minimal optimization required
- High reproducibility
- Stable at 4°C

5. Application

- Multiplex PCR, PCR-based DNA fingerprinting (VNTR, STR, and RAPD) etc.
- Real-time PCR

6. PCR Amplification

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 Table 1) using the water. This option 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 DNA, added at step 4, should be 12.5 µl per reaction.

2. Mix by vortexing briefly, and dispense 12.5 µl into each PCR tube according to Table 1. It is important to mix EzWay™ Multiplex PCR qMasterMix prior to use to avoid localized differences in salt concentration. EzWay™ Multiplex PCR qMasterMix is provided as a 2X concentrate (i.e., a 12.5µl volume of the EzWay™ Multiplex PCR qMasterMix is required for amplification reactions with a final volume of 25 µl). For volumes smaller than 25 µl, the 1/1 ratio of EzWay™ Multiplex PCR qMasterMix to diluted primer mix and template should be maintained as defined in Table 1. A negative control (without template DNA) 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™ Multiplex PCR qMasterMix.

4. Add template DNA to the individual PCR tubes.

For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of the final PCR volume.

Table 1. Reaction composition using EzWay™ Multiplex PCR qMasterMix

Component	**Final Concentration**	**Volume/reaction**	
2X EzWay™ Multiplex PCR qMasterMix	1X	12.5 µL	25 µL
5’ Primer	0.4 uM	Variable	Variable
3’ Primer	0.4 uM	Variable	Variable
(TaqMan) Probe	0.1 - 0.2 uM	Variable	Variable
Distilled water	-	Variable	Variable
Template DNA	Less than 1ug/rx	Variable	Variable
Total reaction volume		**25 µL**	**50 µL**

5. Program the thermal cycler according to the manufacturer’s instructions. A typical PCR cycling program is outlined in Table 2.

In cycling program, pre-heating step (95°C for15 min) should be included to regenerate the activity of Hot Taq DNA Polymerase.
For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

Note : Make sure the PCR mixture is kept away from light

Table 2. Thermal cycler conditions

Step		Temp.	Time	Cycles
Initial Denaturation		95℃	15 min	1
Cycling (3 steps)	Denaturation	94℃	15 sec	35-45 [3)]
	Annealing	50-60℃	30 sec [1)]	
	Extension	72℃	30 sec [2)]	

Or

Step		Temp.	Time	Cycles
Initial Denaturation		95℃	15 min	1
Cycling (2 steps)	Denaturation	94℃	15 sec	35-45 [3)]
	Annealing & Extension	50-60℃	45-90 sec [2)]	

1) Approximately 5-8°C below Tm of primers.
2) Perform fluorescence data collection.
3) The number of cycles depended on the copy number of template DNA or cDNA.