EzWay™ Hot Universal qMasterMix

1. Catalog No.

Cat. No	Product	Size
K0569010	EzWay™ Hot Universal qMasterMix	1ml

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™ Hot Universal qMasterMix	K0569010	1.0ml	Mixture of Hot Taq DNA Polymerase, qPCR Buffer, dNTP and additives
4X Magic Buffer	K0561031	1.0ml	**Only use for High GC content**

4. Description

2X EzWay™ Hot Universal qMasterMix is a premixed solution containing Hot Taq DNA Polymerase, dNTPs and the specialized reaction buffer for probe-based qPCR.
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.
2X EzWay™ Hot Universal qMasterMix guarantees the highly specific and reproducible quantitative results.
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.

- Real-time quantitative PCR of DNA and cDNA targets using fluorescent probe
- High PCR specificity
- High sensitivity
- High reproducibility

5. Application

- Real-Time quantification of DNA and cDNA targets
- Gene expression profiling
- Microbial & Viral pathogen detection

6. PCR Amplification

7. Add the following reagents to a thin-walled PCR microcentrifuge tube or plate. Keep the master mix on ice.

Component	Final Concentration	Volume/reaction	
2X EzWay™ Hot Universal qMasterMix	1X	10 µL	25 µL
5' Primer	0.4 uM	Variable	Variable
3' Primer	0.4 uM	Variable	Variable
Probe	0.1-0.2 uM	Variable	Variable
Distilled water	-	Variable	Variable
Template	Less than 1ug/rx	Variable	Variable
Total reaction volume		**20 µL**	**50 µL**

Note:

It is important to mix 2X EzWay™ Hot Universal qMasterMix before use to avoid localized differences in salt concentration. EzWay™ Hot Universal qMasterMix is provided as a 2X concentrate (i.e., a 25-µl volume of the 2X EzWay™ Hot 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 2X EzWay™ Hot Universal qMasterMix to diluted primer mix and template should be maintained as defined in the table above. 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.

8. Mix gently.

9. Perform thermal cycling by either A or B.

Note:

Program the thermal cycler according to the manufacturer's instructions. The suggested condition is below. The pre-heating step (95°C for 15 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.

Method A

Step		Temp.	Time	Cycles
Initial Denaturation		95℃	15 min	1
Cycling	Denaturation	94℃	15 sec	35-45
	Annealing	50-60℃	30 sec	
	Extension	72℃	30 sec	

Method B

Step		Temp.	Time	Cycles
Initial Denaturation		95℃	15 min	1
Cycling	Denaturation	94℃	15 sec	35-45
	Annealing & Extension	50-60℃	45-90 sec	