

EzWay™ Multiplex PCR MasterMix

1. Catalog No.

Cat. No	Product	Size
K0567810	EzWay™ Multiplex PCR MasterMix (2X)	1ml
K0567815	EzWay™ Multiplex PCR MasterMix (2X)	5ml (1ml x 5)

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	K0567810	K0567815
2X EzWay™ Multiplex PCR MasterMix	K0567810	O (1.0ml)	-
	K0567815	-	O (5.0ml)
4X Magic Buffer (Only use for High G+C content)	K0561031	O (1.0ml)	O (1.0ml)

4. Description

2X EzWay™ Multiplex PCR MasterMix is a premixed solution containing the optimized amount of Hot Taq DNA Polymerase, Multiplex PCR Buffer, MgCl₂, dNTPs and a dye. Room-temperature reaction setup using 2X EzWay™ Multiplex PCR MasterMix is fast and easy. Simply add 1 volume of primers and template DNA diluted in the distilled water into equal volume of 2X EzWay™ Multiplex PCR MasterMix.

Pipetting steps are minimized, reducing the possibility of errors and contamination.

The combination of high specificity and easy handling makes the 2X EzWay™ Multiplex PCR MasterMix ideal for use with complex genomic or cDNA templates, multiple primer pairs, templates isolated from difficult sources, genetic screening in which large numbers of samples are amplified, and laboratory PCR automation.

- Multiplex PCR
- High PCR specificity
- Reduced non-specific amplification
- Simple reaction setup at room temperature
- Minimal optimization required
- High reproducibility

5. Application

- Multiplex PCR, PCR-based DNA fingerprinting (VNTR, STR, and RAPD) etc.
- Highly Specific PCR
- Low Copy Number Target PCR. (e.g. Viral Detection in Blood)
- RT-PCR of rare transcripts
- Differential Display
- Degenerate PCR

6. PCR Amplification

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

Component	Final Concentration	Volume/reaction	
		10 µL	25 µL
2X EzWay™ Multiplex PCR MasterMix	1X	10 µL	25 µL
5' Primer	0.1 - 0.5 µM	Variable	Variable
3' Primer	0.1 - 0.5 µM	Variable	Variable
Distilled water	-	Variable	Variable
Template	Less than 1µg/rx	Variable	Variable
Total reaction volume		20 µL	50 µL

Note:

It is important to mix the EzWay™ Multiplex PCR MasterMix before use to avoid localized differences in salt concentration. Multiplex EzWay™ Multiplex PCR MasterMix is provided as a 2X concentrate (i.e., a 25ul volume of the EzWay™ Multiplex PCR MasterMix is required for amplification reactions with a final volume of 50ul). For volumes smaller than 50ul, the 1/1 ratio of EzWay™ Multiplex PCR MasterMix 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.

2. Mix gently.
3. When using a thermal cycler without a heated lid, add approximately 50ul of mineral oil on top of the mixture.
4. Perform thermal cycling.

Step	Temp.	Time	Cycles
Initial Denaturation	95°C	15 min	1
Cycling	Denaturation	94°C	0.5-1 min
	Annealing	50-68°C	0.5-1 min
	Extension	72°C	1 min (~1kb/imin)
Final Extension	72°C	10 min	1

Note:

- a. Primers should be 15 to 30 bases in length and near 50% G+C content.
- b. **Magic Buffer is not necessary for normal G+C content. It will improve DNA amplification of templates that have a high G+C content and a high degree of secondary structure.** We recommend that the volume added should not exceed 25 % (v/v) of final PCR volume.