

# 2X ACE Hot Start Taq Master Mix

Cat# EP1508 – 1ml | EP1509 – 5ml | EP1510 – 15 ml

Storage: All components should be stored at -20°C.

## INTRODUCTION

This product includes ACE Hot Start Taq DNA Polymerase, dNTPs, and an optimized buffer system that allows for amplification by simply adding primers and templates, reducing pipetting and improving throughput and reproducibility. A protective agent added to the amplification system allows the 2X ACE Hot Start Taq Master Mix to maintain stable activity after repeated freeze-thaw cycles. This product is available in a version containing electrophoresis buffer and dye. It can be directly electrophoresed after the reaction. The 3' end of the PCR product, A, can be directly cloned into the T vector and used in the ACE One Step Cloning Kit series (ACE, Cat.No. #EC1001, #EC1008).

## CONTENTS

Component	EP1508	EP1509	EP1510
2X ACE Hot Start Taq Master Mix	1ml	5*1ml	15*1ml

## UNIT DEFINITION

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-insoluble products in 30 min at 74 °C with activated salmon sperm DNA as the template / primer.

## PROTOCOL

### Reaction system

ddH <sub>2</sub> O	to 50 µl
2X ACE Hot Start Taq Master Mix	25 µl
Primer 1 (10 µM)	2 µl
Primer 2 (10 µM)	2 µl
Template DNA*	X µl

\*The optimal reaction concentration of different templates is different. The following table shows the recommended template usage for 50 µl reaction system.

Human Genomic DNA	1- 500 ng
E.coli Genomic DNA	1 - 100 ng
λ DNA	0.1 - 1 ng
Plasmid DNA	0.1 - 1 ng

## Reaction procedure

95°C	5 min (Pre-denaturation) <sup>a</sup>	
95°C	30 sec	} 30 - 35 cycles
55°C <sup>b</sup>	30 sec	
72°C	60 sec/kb	
72°C	7 min (Complete Extension)	

a. The pre-denaturation time takes at least 5 minutes. If the amplification is not ideal, the pre-denaturation time of 95°C can be extended appropriately, up to 10 min.

b. The annealing temperature needs to be adjusted according to the T<sub>m</sub> value of the primer, and is generally set to be lower than the primer T<sub>m</sub> value of 3 - 5°C.

## PRIMERS DESIGNING NOTES

1. Choose C or G as the last base of the 3'-end of the primer;
2. Avoid continuous mismatching at the last 8 bases of the 3'-end of the primer;
3. Avoid hairpin structure at the 3'-end of the primer;
4. T<sub>m</sub> of the primers should be within the range of 55°C - 65°C;
5. Additional sequence should not be included when calculating T<sub>m</sub> of the primers;
6. GC content of the primers should be within the range of 40% - 60%;
7. T<sub>m</sub> and GC content of forward and reverse primers should be as similar as possible.

## PRODUCT USE LIMITATION

These products are intended for research use only.