# 2X ACE LAmp Master Mix <br> Cat\# EP1407-1ml|EP1408-5*1 ml | EP1409-15*1 ml <br> Storage: All components should be stored at $-20^{\circ} \mathrm{C}$. 

## INTRODUCTION

$2 \times$ ACE LAmp Master Mix is a blend of Taq DNA Polymerase and a DNA proofreading polymerase with $3^{\prime}$ to 5' exonuclease activity. Its fidelity was 6-fold higher than conventional Taq DNA Polymerase. Used with the optimized buffer system, $2 \times$ ACE LAmp Master Mix is applicable to long PCR products, up to 21 kb . This Master Mix is also able to amplify long fragments accurately from templates of different sources or different length.
$2 \times$ ACE LAmp Master Mix contains Vazyme LAmp DNA Polymerase, dNTP, and optimized buffer. The reaction can be started by adding only primers and template, which simplifies the operation, improves through-put, and enhances result reproducibility. The protective agents included guarantees the stability of the activity of this Master Mix. The PCR product, containing dA at 3'-end, can be cloned into T-vector, and is suitable for One Step Express cloning kit.

CONTENTS

| Component | EP1407 | EP1408 | EP1409 |
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| $2 X$ ACE LAmp Master Mix | 1 ml | $5 * 1 \mathrm{ml}$ | $15^{*} 1 \mathrm{ml}$ |

## PROTOCOL

1. General reaction mixture for PCR:

| ddH2O | to $50 \mu \mathrm{l}$ |
| :--- | :--- |
| $2 \times$ ACE LAmp Master Mix | $25 \mu \mathrm{l}$ |
| Template DNA* | Optional |
| Primer $1(10 \mu \mathrm{M})$ | $2 \mu \mathrm{l}$ |
| Primer $2(10 \mu \mathrm{M})$ | $2 \mu \mathrm{l}$ |

*The recommended amount of DNA template for a $50 \mu \mathrm{l}$ reaction system is as follows:

| Human Genomic DNA | $10-200 \mathrm{ng}$ |
| :--- | ---: |
| Bacterial Genomic DNA | $1-100 \mathrm{ng}$ |
| $\lambda$ DNA | $0.1-10 \mathrm{ng}$ |
| Plasmid DNA | $0.1-10 \mathrm{ng}$ |

2. Thermocycling conditions:
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\begin{array}{ll}\hline 94^{\circ} \mathrm{C} & 5 \mathrm{~min} \text { (Pre-denaturation) } \\
94^{\circ} \mathrm{C} & 30 \mathrm{sec} \\
55^{\circ} \mathrm{C} * \\
72^{\circ} \mathrm{C} & 30 \mathrm{sec} \\
72^{\circ} \mathrm{C} & 30 \mathrm{sec} / \mathrm{kb} \\
7 \mathrm{~min} \text { (Final extension) }\end{array}
$$\right\} \begin{array}{l} <br>

\hline\end{array}\right\}\)|  |
| :--- |

*The optimal annealing temperature should be $1-2^{\circ} \mathrm{C}$ lower than the $\mathrm{T}_{\mathrm{m}}$ of the primers used.
$\left.\begin{array}{ll}94^{\circ} \mathrm{C} & 1-3 \mathrm{~min} \text { (Pre-denaturation) } \\ 94^{\circ} \mathrm{C} \\ 68^{\circ} \mathrm{C} * \\ 68^{\circ} \mathrm{C} & 10 \mathrm{sec} \\ 30-60 \mathrm{sec} / \mathrm{kb} \\ 7 \mathrm{~min} \text { (Final extension) }\end{array}\right\} 30-35 \mathrm{cycles}$

* For amplification of a DNA fragment $>5 \mathrm{~kb}$, it is recommended to use long primers which Tm between $68^{\circ} \mathrm{C}$ and 70 ${ }^{\circ} \mathrm{C}$. The temperature for both annealing and extension should be $68^{\circ} \mathrm{C}$, which can significantly improve the amplification specificity. Extending extension time could increase the amplification yield.


## PRIMERS DESIGNING NOTES

1. Choose C or G as the last base of the $3^{\prime}$-end of the primer;
2. Avoid continuous mismatching at the last 8 bases of the $3^{\prime}$-end of the primer;
3. Avoid hairpin structure at the 3 '-end of the primer;
4. $\mathrm{T}_{\mathrm{m}}$ of the primers should be within the range of $55^{\circ} \mathrm{C}-65^{\circ} \mathrm{C}$;
5. Additional sequence should not be included when calculating Tm of the primers;
6. GC content of the primers should be within the range of $40 \%-60 \%$;
7. $\mathrm{T}_{\mathrm{m}}$ and GC content of forward and reverse primes should be as similar as possible.

## PRODUCT USE LIMITATION

These products are intended for research use only.

