

CloneAmp HiFi PCR Premix (Cat. No. 639298) is a convenient 2X master mix that provides exceptionally accurate and efficient DNA amplification, due to the high sensitivity, specificity, priming efficiency, and extension efficiency of CloneAmp HiFi Polymerase. This abbreviated protocol is provided for your convenience.

1. Prepare the Master Mix

- Prepare the Master Mix by adding each component indicated in Table 1.

NOTE: The Master Mix can be prepared at room temperature. However, keep each of the reaction components on ice during the preparation process.

Table 1. Recommended Master Mix.

Reagent	Volume	Final Conc.
CloneAmp HiFi PCR Premix	12.5 µl	1X
Primer 1	5–7.5 pmol	0.2–0.3 µM
Primer 2	5–7.5 pmol	0.2–0.3 µM
Template	< 100 ng	
Sterilized distilled water	up to 25 µl	
Total volume per reaction	25.0 µl	

- Mix the Master Mix by tapping the bottom of the tube, then centrifuge briefly. Transfer 25 µl of the Master Mix into individual PCR tubes or each well of a PCR plate. Centrifuge briefly.

- Program your thermal cycler with the following cycling conditions:

NOTE: When performing rapid amplification protocols using CloneAmp HiFi Polymerase, 3-step reactions are recommended for best results and longest amplification products.

- For reactions in which the amount of template is less than 100 ng:

98°C	10 sec	30–35 cycles
55°C	5 or 15 sec	
72°C	5 sec/kb	

- For reactions in which the amount of template exceeds 100 ng:

98°C	10 sec	30–35 cycles (3-step PCR)
55°C	5 or 15 sec	
72°C	30–60 sec/kb	

or

98°C	10 sec	30–35 cycles (2-step PCR)
68°C	30–60 sec/kb	

CloneAmp™ HiFi PCR Premix Protocol-At-A-Glance

Contact Us For Assistance	
Customer Service/Ordering	Technical Support
Telephone: 800.662.2566 (toll-free)	Telephone: 800.662.2566 (toll-free)
Fax: 800.424.1350 (toll-free)	Fax: 800.424.1350 (toll-free)
Web: www.clontech.com	Web: www.clontech.com
E-mail: orders@clontech.com	E-mail: tech@clontech.com

Notice to Purchaser

Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Clontech Laboratories, Inc.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at <http://www.clontech.com>. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

Clontech, the Clontech logo, and CloneAmp are trademarks of Clontech Laboratories, Inc. All other marks are the property of their respective owners. Certain trademarks may not be registered in all jurisdictions. Clontech is a Takara Bio Company. ©2012 Clontech Laboratories, Inc.

This document has been reviewed and approved by the Clontech Quality Assurance Department.