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For Research Use Only

PCR AZMA Master Mix

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Description: PCR AZMA Master Mix is a premixed, ready-to-use solution containing Taq DNA polymerase, dNTPs, MgCl2 and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR.

Storage Conditions: Product may be stored at **-20°C** for up to **8** months. Minimize the number of freeze and thaw cycles by storing in working aliquots. Mix well prior to use.

Protocol

1. Thaw the **PCR AZMA Master Mix** at room temperature. Vortex the Master Mix and then spin it briefly in a microcentrifuge to collect the material in the bottom of the tube.

2. Prepare the following reaction mixes on ice (For a **20** µl reaction volume)

Component	Volume	Final Conc.
2X PCR Master Mix	10 µl	1X
Forward Primer (10 pmol/ µL)	1 µl	0.5 pmoles/µL
Reverse Primer (10 pmol/ µL)	1 µl	0.5 pmoles/µL
DNA template	Variable	10 fg~1 µg
Nuclease-Free Water	to 20 µl	-

General Guidelines for Amplification by PCR for under 3 Kb target sequence

A. Denaturation

- Generally, a **5**-minute initial denaturation step at **94°C** is sufficient.
- Subsequent denaturation steps will be between **30** seconds and **1** minute.

B. Annealing

- The annealing step is typically **30** seconds to **1** minute.

C. Extension

- Allow approximately **1** minute for every **1kb** of DNA to be amplified.
- A final extension of **5** minutes at **72**°C is recommended.

D. Refrigeration

- If the thermal cycler has a refrigeration cycle, the cycling reaction can be programmed to end by holding the tubes at 4°C for several hours.

E. Cycle Number

- Generally, **25–30** cycles result in optimal amplification of desired products.
- Occasionally, up to 40 cycles may be performed, especially for detection of low-copy targets.

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