UniAccu *Pfu* Green Master Mix (2X)

Instructions for Use of Products ATR-E50911 and ATR-E50912.

ATR MED

Quick Protocol

UniAccu *Pfu* Green Master Mix (2X) contains *Pfu* DNA Polymerase, dNTPs, and a unique buffer system that ensures reliable PCR performance in a wide range of PCR applications without the need for time-consuming optimization. It only requires the addition of primers and templates to perform the amplification reaction, thereby reducing pipetting operations and improving the detection throughput and reproducibility of results. **UniAccu *Pfu* Green Master Mix (2X)** ensures highly specific amplification with different primer–template systems. It is ideally suited for routine PCR applications for DNA amplicons in the range of up to 3kb from templates, including pure DNA solutions, bacterial colonies, and cDNA products. The amplification system contained protective agents that maintained **UniAccu *Pfu* Green Master Mix (2X)** activity after repeated freezing and thawing. This product contains a loading buffer; therefore, PCR products can be directly loaded for electrophoresis after the reaction, which is convenient to use.

Storage

Stored at -20°C.

Protocols

UniTaq Red PCR Master Mix (2X) should be used at a 1X concentration with DNA template and primers in a total reaction volume of 25 µL. The reaction volumes can be scaled up to 50 µL if desired.

1. Gently vortex and briefly centrifuge **UniTaq Red PCR Master Mix (2X)** after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 25 µL reaction:

PCR Master Mix (2X)	12.5 µL
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA*	10 pg - 1 µg
Water, nuclease-free	to 25 µL

***Optimal DNA concentration varies in different templates. In a 25 µL system, the recommended template usage is as follows:**

Template DNA	Concentration
Animal & Plant Genomic DNA	0.1 - 1 µg
E. coli Genomic DNA	10 - 100 ng
cDNA	1 - 5 µl (≤1/10 of the total volume of PCR system)
Plasmid DNA	0.1 - 10 ng
λDNA	0.5 - 10 ng

3. Gently vortex the samples and spin down.
4. Perform PCR using the recommended thermal cycling conditions outlined below:

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation [a]	95°C	1-3 min	1
Denaturation	95°C	30 sec	25-40
Annealing [b]	Tm-5°C	30 sec	
Extension [c]	72°C	2 min/kb	
Final extension [d]	72°C	5-15 min	1

[a] Initial denaturation conditions are applicable for most amplification reactions and can be adjusted according to the complexity of the template structure. If the template structure is complex, the initial denaturation time can be extended to 5 - 10 min to improve its effect.

[b] The annealing temperature needs to be adjusted according to the Tm value of the primer, which is generally set to 3–5 °C lower than the Tm value of the primer; for complex templates, it is necessary to adjust the annealing temperature and extend the extension time to achieve efficient amplification.

[c] For longer products, the extension time should be prolonged by 2 min/kb. For amplification of longer templates (>2 kb) a reduction of the extension temperature to 68°C is required to avoid enzyme inactivation during prolonged extension times.

[d] If the PCR product has to be cloned into a TA vector, the final extension step may be prolonged to 30 min to ensure the highest efficiency of 3'-dA tailing of the PCR product.

5. Load 3-5 µL of PCR mixture directly on a gel.

Additional protocol information is available in Product Information #ATR-E50911 and #ATR-E50912, available online at: www.atrned.com