UniAccu *Pfu* DNA Polymerase (Mg^{2+} Plus Buffer)

Instructions for Use of Products ATR-P515-1

Quick Protocol

Kit Contents

Components	ATR-250 Units
UniAccu *Pfu* DNA Polymerase, 2.5 U/µL	100 µL
10X UniAccu *Pfu* Reaction Buffer with $MgSO_4$	1 mL

Storage

Stored at -20°C.

Protocol

To prepare several parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing water, buffer, dNTPs, primers, and template DNA. **UniAccu *Pfu* DNA Polymerase** should be the last component added. Prepare a sufficient master mix for the number of reactions plus one extra to allow for pipetting error.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 25 µL reaction. Reaction volumes can be scaled up to 50 µL if desired.

Components	Reaction Volume
10X UniAccu *Pfu* Reaction Buffer	2.5 µL
dNTP Mix (10 mM each)	0.5 µL (200 µM)
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA*	10 pg - 1 µg
UniAccu *Pfu* DNA Polymerase (2.5U/µL)	0.5-1 µL (1.25-5 U)
Water, nuclease-free	to 25 µL

[#] 25 mM $MgSO_4$ solution should be added to 25 µL reaction:

Final concentration of $MgSO_4$ (mM)	**1**	**1.25**	**1.5**	**1.75**	**2**	**2.5**	**3**	**4**
Volume of 25 mM $MgSO_4$ (µL)	**1**	**1.25**	**1.5**	**1.75**	**2**	**2.5**	**3**	**4**

***Optimal DNA concentration varies in different templates. 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. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Perform PCR using the recommended thermal cycling conditions outlined below:

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation [a]	95	1-3 min	1
Denaturation	95	30 sec	25-35
Annealing [b]	Tm-5	30 sec	
Extension [c]	72	2 min	
Final extension	72	5-15 min	1

[a] The condition of initial denaturation is 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, generally set to be 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] The recommended extension step is 2 min for PCR products up to 2 kb.

6. 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