Product Information

UniAccu *Pfu* Green Master Mix (2X)

Catalogue Number	Size
ATR-P518-1	1 mL (40 x 25 µl reactions)
ATR-P518-2	5×1 mL (200 x 25 µl reactions)

Product Description

UniAccu Pfu Green Master Mix (2X) contains highly purified and free of bacterial DNA genetically engineered *Pfu* DNA Polymerase, high-quality dNTPs and a unique buffer system that minimizes the requirement for optimization. It only needs to add primers and templates to perform amplification reaction, thereby reducing pipetting operations and improving detection throughput and reproducibility of results

UniAccu *Pfu* Green Master Mix (2X) outperformed *Pfu* Master Mix tested from other suppliers and ensures reliable PCR performance in a wide range of PCR applications without the need for time-consuming optimization. **UniAccu *Pfu* Green Master Mix (2X)** ensures highly specific amplification with different primer-template systems. Every lot of ATR-MED's **UniAccu *Pfu* Green Master Mix (2X)** is subjected to a comprehensive range of quality control tests, including a stringent PCR specificity and reproducibility assay. It is ideally suited to efficient highest fidelity PCR amplifications of DNA amplicons in the range of up to 5-10 kb from templates including vector, genomic DNA, or cDNA. *Pfu* DNA Polymerase exhibits approximately 10 times higher accuracy compared to *Taq* DNA polymerase. *Pfu* DNA Polymerase produces blunt-end PCR products suitable for blunt cloning.

The amplification system contains protective agents that keep **UniAccu *Pfu* Green Master Mix (2X)** in activity after repeated freezing and thawing. **UniAccu *Pfu* Green Master Mix (2X)** can be stored at 2–8°C for up to 2 months, so for frequent use, an aliquot may be kept at 4°C allowing even faster PCR setup by eliminating thawing time. This product contains a loading buffer, so PCR products can be directly loaded for electrophoresis after the reaction, which is convenient to use.

Applications

- **High-fidelity and High-throughput PCR amplification**
- **Blunt-end cloning and site-directed mutagenesis**
- **PCR amplification of GC-rich sequences**

Highlights

- **High yields and specific PCR products with minimal optimization with different primer-template systems**
- **Saves time and reduces contamination**
- **Stable after fifteen freeze-thaw cycles and**
- **Stable for 2 months at 4°C**

Storage

Stored at -20°C.

Shipping

The kit is shipped with ice gel transport at ≤0°C.

Protocols

UniAccu *Pfu* Green Master Mix (2X) should be used at a 1X concentration with DNA template and primers in a total reaction volume of 25 µL. Reaction volumes can be scaled up to 50 µL if desired.

1. Gently vortex and briefly centrifuge **UniAccu *Pfu* Green 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. 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°C	1-3 min	1
Denaturation	95°C	30 sec	25-35
Annealing [b]	Tm-5°C	30 sec	
Extension [c]	72°C	2 min	
Final extension	72°C	5 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℃ 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.

Important Notes

1. Contamination Prevention in PCR Setup

During PCR, where over 10 million copies of template DNA are produced, meticulous care is crucial to prevent contamination from other templates and amplicons present in the laboratory environment. General recommendations to mitigate contamination risk include:

- Perform DNA sample preparation, PCR mixture setup, thermal cycling, and PCR product analysis in separate areas.
- Use a laminar flow cabinet equipped with a UV lamp for PCR mixture preparation.
- Wear fresh gloves for DNA purification and reaction setup.
- Utilize reagent containers solely dedicated to PCR.
- Employ positive displacement pipettes or pipette tips with aerosol filters for DNA sample preparation and PCR setup.
- Ensure the use of PCR-certified reagents, including high-quality water.
- Always include "no template control" (NTC) reactions to monitor for contamination.

2. PCR Primer Design

For PCR primer design, follow the following guidelines:

- PCR primers typically range from 15 to 30 nucleotides in length.
- Maintain a maximum difference of 5°C in melting temperatures (Tm) between the two primers.
- Exclude additional primer sequences not matched with the template when calculating primer Tm.
- Aim for a primer GC content of 40-60%, with uniform distribution of C and G nucleotides.
- Limit the presence of three or more G or C nucleotides at the 3'-end to mitigate non-specific priming.
- Preferentially terminate primers with a G or C at the 3'-end.
- Avoid self-complementary regions and complementarities between primers to prevent hairpin formation and primer dimerization.
- Check for potential undesired primer-template complementary sites.
- When designing degenerate primers, ensure at least 3 conserved nucleotides at the 3'-end.
- Refer to primer design tools for efficient introduction of restriction enzyme sites into primers.
- Use NCBI BLAST to assess primer specificity and prevent nonspecific amplification.

3. PCR Reaction Mixture Components

Components of the PCR reaction mixture, including template DNA, primers, $MgCl_2$ concentration, and dNTPs, require careful consideration:

Template DNA

Higher template amounts increase the risk of non-specific PCR product formation, while lower amounts compromise amplification accuracy. Trace contaminants from DNA purification agents like phenol, EDTA, and proteinase K can inhibit DNA polymerases; ethanol precipitation and repeated washes with 70% ethanol can remove these contaminants.

Primers

The optimal concentration range for PCR primers is 0.1-1 µM. Excessive primer concentrations increase the risk of mispriming and non-specific PCR product formation. For degenerate and long PCR primers, higher concentrations ranging from 0.3-1 µM are recommended.

$MgSO_4$ Concentration

Optimal Mg^{2+} concentration is vital for maximizing PCR yield due to its binding to dNTPs, primers, and DNA templates. The recommended range is 1-4 mM. Insufficient Mg^{2+} levels may decrease PCR product yield, while excessive concentrations can lead to the formation of non-specific products and reduced PCR fidelity. For standard PCR with Taq DNA Polymerase and 0.2 mM dNTPs, the recommended $MgSO_4$ concentration typically falls within 1.5±0.5 mM. In cases where DNA samples contain EDTA or other metal chelators, adjust the Mg^{2+} ion concentration in the PCR mixture accordingly, as each molecule of EDTA binds one Mg^{2+} ion.

dNTPs

The recommended final concentration for each dNTP is 0.2 mM. In certain PCR applications, higher dNTP concentrations may be required. Adjust the $MgCl_2$ concentration accordingly due to Mg^{2+} binding to dNTPs. It is crucial to maintain equal concentrations of all four nucleotides (dATP, dCTP, dGTP, and dTTP) in the reaction mixture.

4. PCR Cycling Parameters

Initial DNA denaturation and enzyme activation

Complete denaturation of template DNA at the onset of PCR is crucial for efficient template utilization during the initial amplification cycle. For templates with ≤50% GC content, a 1-3 minute denaturation at 95 °C suffices. A denaturation time of 30 seconds per cycle at 95 °C is generally adequate, though for GC-rich templates, extending this to 3-4 minutes may be necessary.

Primer annealing

The annealing temperature should be set 5 °C below the melting temperature (Tm) of the primers. A 30-second annealing period is typically adequate. Incremental optimization of annealing temperature in 1-2°C steps may be necessary if non-specific PCR products arise.

Extension

The optimal extension temperature for *Pfu* DNA polymerase is 70-75 °C. The recommended extension step is 2 min/kb at 72°C for PCR products up to 2 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.

Number of cycles

For reactions with <10 template copies, approximately 40 cycles are needed. 25-35 cycles suffice for reactions with higher template amounts.

Final extension

Following the final cycle, it is advisable to incubate the PCR mixture at 72 °C for an additional 5-15 minutes to allow for the completion of any potentially incomplete reaction products.

Notes

Pfu DNA Polymerase exhibits reactivity at room temperature. Therefore, it is recommended to prepare the PCR system on ice before transferring it to the PCR machine for the reaction. This practice minimizes non-specific amplification that may occur during the reaction preparation stage, enhancing the specificity of amplification results. Additionally, avoid using dUTP, dITP, and primers containing these nucleotides in PCR with *Pfu* DNA Polymerase, as the binding of this enzyme to DNA templates containing uracil and hypoxanthine can halt DNA synthesis.

Precautions and Disclaimer

This product is intended for research and development (R&D) purposes only and is not suitable for use in drugs, diagnostic procedures, households, or other applications. When handling the product, always wear appropriate laboratory

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