Product Information

UniMax *Taq* Plus Master Mix (2X)

Catalogue Number	Size
ATR-P511-1	1 mL (40 x 25 µl reactions)
ATR-P511-2	5×1mL (200 x 25 µl reactions)

Product Description

UniMax Taq Plus Master Mix (2X) is a mixture of Taq DNA polymerase and an enzyme containing 3'→5' exonuclease activity. Its fidelity is 6 times greater than that of Taq DNA Polymerase. Compared with Taq DNA Polymerase, Taq Plus DNA polymerase has stronger amplification performance, higher sensitivity, and is more tolerant of impurities within 5 kb amplifying range.

It only needs to add primers and templates to perform amplification reaction, thereby reducing pipetting operations and improving detection throughput and reproducibility of results

Applications

- **High throughput PCR**
- **Routine PCR with high reproducibility**

Highlights

- **Highly specific amplification with different primer–template systems**
- **Stable after fifteen freeze-thaw cycles**
- **Stable for two months at 4°C**

Storage

Stored at -20°C.

Shipping

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

Protocols

UniMax Taq Plus 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 if desired.

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

UniMax Taq Plus 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	95	1-3 min	1
Denaturation	95	30 sec	25-40
Annealing	Tm-5	30 sec	
Extension	72	1 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℃ 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 1 min for PCR products up to 1 kb. For longer products, the extension time should be prolonged by 1 min/kb.

6. Load 3-5 µL of PCR mixture directly on a gel. The red tracer co-migrates with 125 bp fragment in a 1% agarose gel. If a more intense tracking dye is desired, an unused lane can be used to run any common tracking dye.

Important Notes

1. Avoiding contamination during PCR setup

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas. Set up PCR mixtures in a laminar flow cabinet equipped with a UV lamp. Wear fresh gloves for DNA purification and reaction setup. Use reagent containers dedicated for PCR. Use positive displacement pipettes, or pipette tips with aerosol filters to prepare DNA samples and perform PCR setup. Always perform “no template control” (NTC) reactions to check for contamination.

2. PCR primer design

Use primer design software or follow the general recommendations for PCR primer design as outlined below: PCR primers are generally 15-30 nucleotides long. Differences in melting temperatures (Tm) between the two primers should not exceed 5 °C. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value. The optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer. Avoid placing more than three G or C nucleotides at the 3’-end to lower the risk of non-specific priming. If possible, the primer should terminate with a G or C at the 3’-end. Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization. Check for possible sites of undesired complementary between primers and template DNA. When designing degenerate primers, place at least 3 conserved nucleotides at the 3’-end. When introducing restriction enzyme sites into primers, refer to the “Primer Design for Restriction Enzyme Cloning” located on www.neb.com to determine the number of extra bases required for efficient cleavage. Use the NCBI BLAST function to check the specificity of the primer to prevent nonspecific amplification.

3. Components of the PCR reaction mixture

Template DNA

Higher amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification. Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol usually remove trace contaminants from DNA samples.

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products. For degenerate primers and primers used for long PCR we recommend higher primer concentrations in the range of 0.3-1 µM.

4. PCR Cycling Parameters

Initial DNA denaturation and enzyme activation

It is essential to completely denature the template DNA at the beginning of PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95 °C is sufficient. Denaturation A DNA denaturation time

of 30 seconds per cycle at 95 °C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min.

Primer annealing

The annealing temperature should be 5 °C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments.

Extension

The optimal extension temperature for Taq DNA polymerase is 70-75 °C. The recommended extension step is 1 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

If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.

Final extension

After the last cycle, it is recommended to incubate the PCR mixture at 72 °C for an additional 5-15 min to fill in any possible incomplete reaction products. 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.

Notes

Operation Taq DNA Polymerase has a certain reactivity at room temperature, please prepare the PCR system on ice, and then place it on the PCR machine for the reaction. This can reduce non-specific amplification that occurs during the reaction preparation stage, and help to obtain highly specific amplification results.

Precautions and Disclaimer

This product is for R&D use only, not for use in drug, diagnostic procedures, household, or other uses. When working with the product, always wear a suitable lab coat and disposable gloves, protective eyewear. When radioactive tracers are used, standard procedures for safely handling radioactive materials should be followed. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@atrmed.com). To the extent allowed by law, ATR-MED Inc. will not be liable for special, incidental, indirect, punitive, multiple, or consequential damages in connection with or arising from this document, including your use of it. By use of this product, you accept all the terms and conditions of ATR-MED products. All trademarks are the property ATR-MED unless otherwise specified.

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