

Product Overview

2× FastAmpli All-in-One qPCR Master Mix with UNG-V4 provides components, e.g. rapid Taq DNA polymerase, uracil DNA glycosylase (UNG), MgCl₂, dNTPs (with dUTP instead of dTTP), and stabilizers, for quantitative PCR (qPCR). With the genetically modified rapid amplification DNA polymerase, it is possible to complete PCR amplification within 30 minutes. The rapid DNA polymerase has also been novelly modified to obtain a hot start version. It blocks polymerase activity at ambient temperatures, and thus prevents extension of nonspecifically annealed primers. This reagent is not only suitable for regular procedures of conventional fluorescent quantitative PCR instruments to obtain a good standard curve in a wide quantitative range, but also suitable for rapid PCR amplification instruments for high-sensitivity and rapid amplification. This reagent uses proprietary reaction buffer for qPCR with well-optimized configuration and UNG/dUTP system, and UNG prevents amplification of carryover PCR products. This reagent is compatible with most fluorescence quantitative PCR instruments from manufacturers such as Applied Biosystems, Eppendorf, Bio-Rad and Roche.

Reagent Composition

2× FastAmpli All-in-One qPCR Master Mix with UNG-V4 contains rapid Taq DNA polymerase, PCR Buffer, UNG, dNTPs (with dUTP instead of dTTP), MgCl₂, stabilizer and other components.

Storage Conditions

Long-term storage at $-20 \pm 5^{\circ}\text{C}$. Mix thoroughly before use and avoid repeated freeze-thaw cycles.

Quality control

1. Amplification sensitivity, specificity, and stability.
2. No exogenous nuclease activity: no exogenous endonuclease and exonuclease pollution.

PCR Reaction System Preparation

Component	Volume per	Volume per	Final Conc.
2× FastAmpli All-in-One qPCR Master Mix with UNG-V4	12.5 μL	25 μL	1×
25× Primer-Probe Mix ¹	1 μL	2 μL	1×
Sample	—	—	—
ddH ₂ O	To 25 μL	To 50 μL	—

1. The final concentration of primer is usually 0.2 μM. For better results, the primer concentration can be optimized within the range of 0.2-1 μM. Generally, the probe concentration can be optimized within the range of 0.1-0.3 μM.
2. When using a fast PCR procedure, increasing the concentration of primers and probes may result in better amplification results, and their ratio should be optimized accordingly.
3. Different types of samples contain different types and contents of inhibitor and copy number of target gene. The sample volume should be considered by actual condition. Make a dilution of the sample with nuclease-free water or TE Buffer, if necessary.

Reaction Conditions

Typical PCR Program				Fast PCR Program			
Procedure	Temp.	Time	Cycle	Procedure	Temp.	Time	Cycle
Digestion	50°C	2min	1	Reverse	50°C	2min	1
Denaturation	95°C	1-5 min	1	Denaturation	95°C	30s	1
Denaturation	95°C	10-20 s	40-45	Denaturation	95°C	1-3 s	40
Annealing and Elongation	56-64°C	20-60 s		Annealing and Elongation	56-64°C	3-20 s	

Technical Info.

1. Amplification rate of rapid DNA polymerase is no less than 1kb/10s. Different PCR instruments have different heating and cooling speeds, temperature control modes and thermal conductivity, thus optimization of your primer/probe concentration and running

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2025V01



method in combination with your specific fast PCR instrument is essential.

2. This product performs wide applicability, and it is suitable for high-sensitivity molecular diagnosis.
3. Three-step PCR method is recommended for primers with low annealing temperature or for amplification of long fragments over 200 bp.
4. Since different amplicons have different utilization efficiency of dUTP and different sensitivity to UNG, the reagents should be optimized if the detection sensitivity decreases when using UNG system. Please contact us for technical support if needed.
5. To avoid amplification of carryover PCR products, dedicated experimental area and pipette are required for amplification. Operate with gloves and change frequently and do not open the PCR tube after amplification.