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FastAmpli Lyo-Ready All-in-One qPCR Master Mix with UNG-V4

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BiOri BIOTECH

Product Overview

FastAmpli Lyo-Ready All-in-One qPCR Master Mix with UNG-V4 is specially developed for the lyophilization process. It 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. After lyophilization, the reagent maintains good stability.

Reagent Composition

FastAmpli Lyo-Ready All-in-One qPCR Master Mix with UNG-V4 contains rapid Taq DNA polymerase, PCR Buffer, UNG, dNTPs (with dUTP instead of dTTP), $MgCl_2$, stabilizer and lyoprotectants.

Storage Conditions

Long-term storage at -20±5℃. 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.
FastAmpli Lyo-Ready 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_2O	To 25 μL	To 50 μL	——

1. This system can be lyophilized and already contains lyoprotectant ingredients.
2. Typically 0.2 μM primer concentration yields better results; if reaction performance is poor then adjust primer concentration between 0.2~1 μM range accordingly. Typically probe concentrations are optimized within the range of 0.1~0.3 μM. Concentration gradient experiments may be performed to find the best combination of primers and probes.
3. 3When 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.
4. The copy number of target genes contained in different types of templates differs; therefore, it may be necessary to perform gradient dilution experiments to determine the optimal amount of template addition.

Reaction Conditions

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

When the system is used for lyophilization, prepare the system as following

Component	Volume per Reaction
FastAmpli Lyo-Ready All-in-One qPCR Master Mix with UNG-V4	12.5 μL
25×Primer-Probe Mix	1 μL
ddH_2O	18~20 μL

Lyophilization Method

Procedure	Temp.	Time	Condition	Pressure
Freezing	4°C	30 min	Hold	1 atm
	-50°C	60 min	Ramp	
	-50°C	180 min	Hold	
Primary Drying	-30°C	60 min	Ramp	Ultimate Vacuum
	-30°C	720 min	Hold	
Secondary Drying	25°C	60 min	Ramp	Ultimate Vacuum
	25°C	300 min	Hold	

*This cycle protocol is suitable for in-situ lyophilization. If you need to produce lyophilized microspheres, please contact us for technical support. The parameters provided are for reference only and should be optimized to different lyophilized product formats and machines.

Guidelines for lyophilized products (In PCR tube)

1. Centrifuge the lyophilized powder instantaneously.
2. Add the template to the lyophilized powder, and add water up to 25 μL.
3. Mix and centrifuge, and then proceed with PCR amplification

* It is recommended to use Biori integrated mixer-centrifuge CM-8 or CM-8 Plus for standardized mixing and centrifugation operations.

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 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 to 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.

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