Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Multiplex PCR MasterMix (UNG)

Product Number: PCM34

Shipping and Storage

-20°C, try to avoid repeated freezing and thawing..

Components

Component	PCM34 1mL	PCM34 5mL
2×Multiplex PCR MasterMix (UNG)	1mL	5×1mL
ddH_2O	1mL	5×1mL

Description

2×Multiplex PCR MasterMix (UNG) is a PCR premix system composed of Golden Star Taq DNA Polymerase, Mg^{2+}, dNTPs (including dUTP), UNG enzyme and PCR stabilizer. The use of this product does not require the optimization of complicated PCR reaction conditions, and multiple PCR reactions can be easily performed by simply exploring the conditions.

The Golden Star Taq DNA Polymerase contained in this product is a chemically modified hot-start enzyme, which can effectively reduce the non-specific amplification caused by primer mismatch in the early stage of PCR reaction. The unique buffer system enables all primers in the multiplex PCR reaction to be effectively extended without additional optimization. Also included in this MasterMix is the GC Enhancer, which facilitates efficient amplification of "difficult" templates (eg, those with high GC content). This product uses the dUTP-UNG anti-pollution system, which can effectively remove the residual pollution of PCR products and greatly reduce the false positives caused by the pollution of amplification products. The UNG enzyme can be inactivated by the pre-denaturation step in the PCR cycle, so it will not affect the formation of new dU-containing PCR products.

Multiplex PCR MasterMix (UNG) can effectively prevent residual contamination of PCR products, and is suitable for anti-pollution multiplex PCR reactions, such as microsatellite analysis, genotyping and SNP detection, etc.

Quality Control

After testing, there is no exogenous nuclease activity; PCR method detects no host residual DNA; storage at 2-8°C for 3 days, no significant change in amplification performance.

Protocol

The following protocol is an example of conventional PCR reaction system and condition. The actual protocol should be improved and optimized based on the template, primer structure and the size of the target.

1. PCR reaction system:

Reagent	50μL	Final Conc.
2×Multiplex PCR MasterMix (UNG)	25μl	1×
Primer Mix，10 μM each	1μl	0.2μM
Template DNA	appropriate amount	
ddH_2O	up to 50μl	

Note: When designing primers, try to minimize the difference in Tm between each primer and keep the difference within 5°C as much as possible,Please use the final concentration of 0.05-0.2μM as a reference for setting the range for each primer concentration.In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific amplification occurs, the primer concentration can be reduced to optimize the reaction system.

2. PCR reaction program:

Step	Temperature	Time	
UNG enzyme digestion	50℃	2-10 min	
Pre denaturation	95℃	10 min	
Denaturation	95℃	30 s	30-40cycles
Annealing	55-65℃	30 s	
Extend	72℃	60 s / kb	
Final extension	72℃	5 min	

Note: 1)In general experiments, the annealing temperature is 5℃ lower than the melting temperature Tm of the amplification primer, and when ideal amplification efficiency cannot be achieved, the annealing temperature should be appropriately reduced; When a non-specific reaction occurs, increase the annealing temperature to optimize the reaction conditions.

2)The extension time should be set based on the size of the amplified fragment, and the amplification efficiency of the Golden Star Taq DNA Polymerase included in this product is 1kb/min.

3)The number of cycles can be set based on the downstream application of the amplification product. If the number of cycles is too small, the amplification amount is insufficient; If there are too many cycles, the probability of mismatch will increase, and the non-specific background will be severe. Therefore, while ensuring product yield, the number of cycles should be minimized as much as possible.

3. Result detection: This product does not contain dyes. After the reaction is completed, take 5μl of the reaction product and add an appropriate amount of sample buffer solution for electrophoresis detection results.