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Super Multiplex PCR Mix (UNG), Lyophilized

Product Number: PCM355

Shipping and Storage

-20±5°C,try to avoid repeated freeze-thaw cycles.

Components

Component	PCM355 1ml	PCM355 5ml
2.5×Super Multiplex PCR Mix (UNG), Lyophilized	1ml	5ml
ddH_2O	1ml	5ml

Description

Super Multiplex PCR Mix (UNG), Lyophilized is a premixed system suitable for various types of multiple PCR, with a concentration of 2.5×,Contains components such as DNA polymerase, UNG enzyme, PCR buffer, dNTPs, Mg^{2+}, and enhancers.

Super Multiplex PCR Mix (UNG), a genetically modified recombinant enzyme containing DNA polymerase, has 5'→3' DNA polymerase activity and no 5'→3' exonuclease activity; DNA polymerase, modified by a new type of antibody, is an antibody modified hot start enzyme with high amplification efficiency. It also has excellent characteristics such as short activation time, strong amplification ability, and high sensitivity.This product introduces a dUTP/UNG anti pollution system, which can effectively remove residual contamination of PCR products and greatly reduce false positives caused by amplification product contamination.

Super Multiplex PCR Mix (UNG) is suitable for anti pollution multiple PCR reactions, such as microsatellite analysis, genotyping, SNP detection, etc. This product can be paired with freeze-drying protectants for the preparation of freeze-drying reagents.

Notes

1. Before use, please gently mix the product upside down after it has completely melted, and centrifuge briefly before use.
2. Avoid repeated freeze-thaw of this product. Repeated freeze-thaw may cause a decrease in product performance, and it is recommended to store it separately.

Protocol

The following examples are the conventional PCR reaction system and reaction conditions. In practical operation, corresponding improvements and optimizations should be made based on different purposes, templates, primer structures, target fragment sizes, and amplification effects.

1. Add 2.5×Super Multiplex PCR Mix (UNG), Lyophilized, 5×Multiplex freeze-dried protective agent, primer probe, template melted and stored on ice for future use.
2. PCR reaction system

Reagent	25μL	50μL	Final Conc.
2.5×Super Multiplex PCR Mix (UNG), Lyophilized	10μL	20μL	1×
Primer Mix	XμL	XμL	
5×Multiplex lyoprotectant	5μL	10μL	1×
Template DNA	XμL	XμL	
ddH_2O	Up to 25μL	Up to 50μL	

Note:When designing primers, the difference in Tm between each primer should be minimized as much as possible, and the difference should be controlled within 5℃ as much as possible. 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. To achieve the optimal amplification effect, it is recommended to use the primer mixture after a brief 10 second vortex oscillation before centrifugation.

3. Mix well, centrifuge briefly, and collect the solution to the bottom of the tube.
4. PCR reaction program

Step	Temperature	Time	cycles
UNG digestion	50℃	2-10 min	1
Pre denaturation	95℃	30 s-5 min[1)]	1
Denaturation	95℃	10 s	30-40
Annealing	55-65℃[2)]	30 s	
Extend	72℃	1kb/min	
Final extension	72℃	5 min	1

Note:1)This product can be pre denatured at 95℃ for 30 seconds to activate the enzyme; complex templates can extend the pre denaturation time to 5 minutes

2)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.

Freeze drying protocol

Stage	Step	Temperature	Slope time	Temperature control time	vacuum degree Pa
Precooling	1	0℃	5 min	30 min	--
Pre freezing	2	-45℃	90 min	300 min	--
Sublimation drying	3	-30℃	90 min	180 min	14 Pa
	4	-10℃	120 min	180 min	14 Pa
	5	0℃	60 min	160 min	14 Pa
Analytical drying	6	30℃	150 min	240 min	14 Pa

1. Requirements for freeze-drying equipment:

Cold trap coil surface temperature ≤ -50℃

Plate layer temperature ≤ -45 ℃, temperature uniformity ± 1℃

Can perform pressure rise test (leak rate test before freeze-drying production)

2. Environmental requirements: Solution packaging and configuration should be carried out under ten thousand level laminar flow protection as much as possible. Dust from the environmental space falls into the solution and becomes crystal nuclei during the freeze-drying process, affecting the supercooling of the solution crystallization and resulting in inconsistent product quality.
3. The temperature and humidity of the outbound environment should be controlled, and it is recommended that the outbound temperature be between 15-25℃ and the humidity be ≤ 30%.