Version: 03/2024

Lyophilization procedure

Stage	Steps	Temperature	Slope Time	Temperature Control Time	Vacuum Degree Pa
Pre-cooling	1	0 ℃	5 min	30 min	--
Pre-freeze	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. Lyophilization equipment requirements:
   The surface temperature of the cold trap coil ≤-50 ℃
   Layer temperature≤ -45 ℃, temperature uniformity±1 ℃
   Can undergo pressure rise test (leak rate test before freeze-dried production)
2. Environmental requirements: solution dispensing and configuration should be carried out under the protection of 10,000-level laminar flow as much as possible, because the dust in the environmental may fall into the solution to become the crystal nucleus of the lyophilization process, which affects the supercooling degree of the crystallization of the solution and leads to inconsistent product quality.
3. The temperature and humidity of the warehouse should be controlled. It is recommended that the temperature of the warehouse should be 15~25 ℃, and the humidity is recommended to be ≤ 30%.

SuperPro Lyo Multiplex PCR Mix (UNG)

Cat. No. : CW3355S（1 mL）
CW3355M（5 mL）

Storage Condition: -20±5 ℃, avoid repeated freeze-thaw.

Components

Component	CW3355S 1 mL	CW3355M 5 mL
2.5×SuperPro Lyo Multiplex PCR Mix (UNG)	1 mL	5 mL
ddH_2O	1 mL	5 mL

Introduction

SuperPro Lyo Multiplex PCR Mix (UNG) is a premixed system suitable for various types of multiplex PCR, with a concentration of 2.5x. It contains DNA polymerase, UNG, PCR Buffer, dNTPs, Mg^{2+}, and enhancer and other components.

The DNA polymerase contained in SuperPro Lyo Multiplex PCR Mix (UNG) is a genetically engineered recombinant enzyme with 5'-3'DNA polymerase activity and no 5'-3' exonuclease activity. DNA polymerase modified by novel antibody has the advantages of short activation time, strong amplification ability, and high sensitivity. The dUTP/UNG anti-contamination system is introduced in this product, which can effectively remove the residual contamination of PCR products and greatly reduce the false positive caused by the contamination of amplification products.

SuperPro Lyo Multiplex PCR Mix (UNG) is suitable for various types of multiple PCR, such as microsatellite analysis, amplicon library preparation, genotyping and SNP detection. This product can be used for the preparation of lyophilized reagents with lyophilization protective agents.

Notes

1. Before use, please gently mix the product upside down after it is completely thawed, and use it after a short centrifugation.
2. Avoid repeated freeze-thaw, which may degrade product performance. It is recommended to store this product in small packages.

Protocol

The following examples show the conventional reaction system and reaction conditions, which should be improved and optimized according to the different templates, primer structures, target fragment sizes and amplification effects.

1. Thaw 2.5×SuperPro Lyo Multiplex PCR Mix (UNG), 5×Multiplex lyophilization protective agents, primers, probe and template on ice.
2. PCR reaction system

Reagents	25 μL System	50 μL System	Final Concentration
2.5×SuperPro Lyo Multiplex PCR Mix (UNG)	10 μL	20 μL	1×
Primer Mix	X μL	X μL	
5×Multiplex lyophilization protective agents	5 μL	10 μL	1×
Template DNA	X μL	X μL	
ddH_2O	to 25 μL	to 50 μL	

Notes:

1)5×Multiplex lyophilization protective agents can be supplied by contacting CoWin's sales on request.

2)When designing primers, the difference between the Tm of each primer should be minimized, and the difference should be controlled within 5 ℃. The primer concentration can be increased when the amplification efficiency is not high, and the primer concentration can be reduced in the case of non-specific reactions, so that the reaction system can be optimized. In order to achieve the best amplification effect, it is recommended that the primer mixture be used after vortex for 10 s and short centrifugation.

3. Mix, 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	
Extension	72 ℃	1 kb/min	
Terminal Extension	72 ℃	5 min	1

Note:

1)The enzyme can be activated with pre-denaturation at 95 ℃ for 30s. For complex template, the pre-denaturation time can be extended to 5 min.

2)In general experiments, the annealing temperature is 5 ℃ lower than the melting temperature (Tm) of the amplification primer, and the annealing temperature should be appropriately reduced when the ideal amplification efficiency cannot be obtained. When nonspecific reactions occur, increase the annealing temperature to optimize reaction conditions.