Tinzyme Co., Limited

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2×Multiplex Pro Mixture

Product Number: PCM05

Shipping and Storage

-20℃, if frequently used, store at 2-8℃.

Components

Component	PCM05 1ml	PCM05 5×1 ml
2×Multiplex Pro Mixture	1 ml	5×1ml
ddH_2O	1 ml	5×1ml

Description

The 2×Multiplex Pro Mixture is an efficient premix composed of a brand new fast and efficient hot start enzyme, unique PCR Buffer, dNTPs, and enhancers and stabilizers. It is mainly used for multiple PCR amplification. This product only requires the addition of primers and templates for amplification, which is simple and convenient to operate, reduces the probability of contamination, improves detection flux and reproducibility.

Based on the hot start polymerase specifically designed for ultra multiplex PCR, compared with existing multiplex reagents, it greatly enhances its amplification ability and anti inhibition ability, and improves the uniformity of amplification. At room temperature, there is no polymerase activity, effectively inhibiting non-specific annealing of primers and non-specific amplification caused by primer dimers under low temperature conditions, ensuring amplification specificity and sensitivity. The unique PCR buffer system significantly improves the amplification efficiency of PCR.

This product is particularly suitable for multiple amplification of gDNA, FFPE DNA, cfDNA and other samples starting from 1-100ng in second-generation sequencing library construction.

Note

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 templates, primer structures, and target fragment sizes.

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 templates, primer structures, and target fragment sizes.

1. PCR reaction system

Reagent	20μL
2×Multiplex Pro Mixture	10μl
Primer Mix	0.1-0.3μM
Template DNA	appropriate amount
ddH_2O	Up to 20μl

2. PCR reaction program:

Step	Temperature	Time
Pre denaturation	98℃	2 min
denaturation	98℃	15 s
Annealing extension	60℃	4 min/8 min/12 min

preserve	4℃	∞

Refer to the table below for the number of PCR cycles and annealing extension time

Multiplicity of single tube primers	Recommended number of cycles (10ng DNA，3,000 copies)		Annealing extension time（min）
	Normal source DNA	FFPF/cfDNA	
10-50	20-22	23-25	4
50-200	18-20	21-23	4
200-800	16-18	19-21	4
Above 800	14-16	17-19	8

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 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. So, while ensuring product yield, the number of cycles should be minimized as much as possible.

3)PCR products are prone to aerosol contamination, leading to inaccurate and unreliable experimental results. It is recommended to physically isolate the PCR reaction system preparation area and PCR reaction area, use specialized pipettes and other equipment, and regularly clean each experimental area (using 0.5% sodium hypochlorite or 10% bleach for wiping and cleaning) to ensure the reliability of the experimental results.