Tinzyme Co., Limited

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One Step RT-qPCR (UNG) Kit, Lyophilized

Product Number:PCK45

Shipping Condition

-20℃, try to avoid repeated freeze-thaw cycles as much as possible

Components

Component	PCK45 200rxns
One Step RT-qPCR (UNG) Kit, Lyophilized(Exo-),5U/μL	320μL
4×SuperPlus Multiplex PCR Buffer	1mL
ddH_2O	1mL

Description

This product is a kit suitable for various types of multiplex PCR, including DNA polymerase and corresponding PCR buffer.

One Step RT qPCR (UNG) Kit, Lyophilized (Exo-) is a genetically engineered recombinant enzyme with 5'→ 3' DNA polymerase activity and no 5'→ 3' exonuclease activity; DNA polymerase, modified by a novel antibody, is a hot start enzyme that can effectively reduce non-specific amplification caused by non-specific binding of primers and templates or primer dimers at room temperature. It also has excellent characteristics such as short activation time, strong amplification ability, high sensitivity, and good stability. The unique combination of PCR buffer system and hot start enzyme significantly improves the amplification efficiency of PCR, with higher sensitivity and stronger inhibitor tolerance.

This product has a wide range of applications and is suitable for various types of multiplex PCR, such as amplicon library construction, microsatellite analysis, gene typing, and SNP detection.

Note

1. Before use, please gently mix the product upside down after it has completely melted, and centrifuge briefly before use.
2. Avoid repeated freezing and thawing of this product, as repeated freezing and thawing may lead to a decrease in product performance. This product can be stored at -20℃ for a long time.

Protocol

The following examples are the STR detection reaction system and reaction conditions. In practical operation, corresponding improvements and optimizations should be made based on specific uses, templates, primer structures, target fragment sizes, and amplification effects.

1. PCR reaction system

Reagent	20μLSystem	Final Conc
One Step RT-qPCR (UNG) Kit, Lyophilized(Exo-),5U/μL	1.6μL	1×
4×SuperPlus Multiplex PCR Buffer	5μL	
Primer Mix[1)]	XμL	1×
Template DNA	XμL	
ddH_2O	Up to 20μL	

Note:1)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 primer concentration 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.

2)During the operation process, human genome contamination should be avoided, and it is recommended to set up a set of negative controls (without DNA) during the experiment.

2. PCR reaction program

Step	Temperature	Time	Cycles
Pre denaturation	95℃	30 s-2min	1
Denaturation	95℃	10s	
Annealing and Extension	55-65℃[1)]	90-150s[2)]	27-31[4)]
Final extension	60℃	10-40min[3)]	

Note: 1) It is recommended to use a two-step PCR reaction program. If good experimental results cannot be obtained due to low Tm values of primers or significant differences in Tm values between primers, three-step PCR amplification can be attempted. The annealing temperature should be set within the range of 55℃-65℃ as a reference (annealing temperature is usually 5℃ lower than Tm value), and the extension temperature should be set at 72℃.

2) When good amplification effect is not achieved, annealing and extension time can be appropriately extended to 120s-150s.

3) When the PCR product detection shows incomplete addition of A, the final extension time can be appropriately extended to 30-40 minutes.

4) The number of cycles can be set based on the downstream application of the amplification product. If the number of cycles is too small and the amplification amount is insufficient, the recommended number of cycles is 27-31.

5) When using the ABI 9700 thermal cycler, please perform amplification in MAX mode.

6) PCR products can be stored at 2-8℃ for short-term storage or at -20℃ for long-term storage.