Tinzyme Co., Limited

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

Animal Detection Probe Mixture (UNG), Lyophilized

Product Number:PCM21

Shipping and Storage

Store at 4-30℃, and store at -20℃ until fully used after re dissolution, avoiding repeated freeze-thaw as much as possible..

Components

Component	PCM21 48rxns	PCM21 100rxns
Animal Detection Probe Mixture (UNG), Lyophilized	6×8 well strip	1×cillin bottle

Description

Animal Detection Probe Mixture (UNG), Lyophilized is a specialized in situ full component freeze-drying reagent suitable for detecting DNA viruses using probe method. It includes novel antibody modified Taq DNA polymerase, PCR Buffer, dNTPs, Mg^{2+}, as well as enhancers and stabilizers.Convenient and fast to use, simply add primer probes and extract the nucleic acid samples for machine amplification. This product is compatible with single and multiple probe qPCR reaction systems.

This product uses the dUTP-UNG anti pollution system, and dUTP is added during the preparation process of the PCR reaction system, resulting in the formation of amplification products containing dU bases.And this product can be eliminated by UNG enzyme treatment in the PCR system before the next PCR reaction.This effectively removes residual contamination of PCR products and greatly reduces false positives caused by amplification product contamination.The pre denaturation step of UNG enzyme in the PCR cycle can be inactivated, so it will not affect the formation of new dU based PCR products.

Note

1. This product can be stored at 4-30℃ for a long time, and at -20℃ for longer periods of storage. If it cannot be used up after re dissolution, it can be stored at -20℃ to avoid repeated freeze-thaw.
2. ROX dye is used to correct the fluorescence signal error generated between quantitative PCR wells, and this product does not contain ROX dye.

PCR reaction system for freeze-drying Well strip

Reagent	25μL PCR reaction	Final Concentration
Animal Detection Probe Mixture (UNG), Lyophilized	1 hole	
ddH_2O	12.5μL	
Primer probe mix[1)]	XμL	
Template DNA[2)]	5μL	
50×ROX reference dye(optional)[3)]	0.5μL	1x
ddH_2O	Up to 25μL	

PCR reaction system for freeze-drying cillin bottles

Reagent	25μL PCR reaction	Final Concentration
Animal Detection Probe Mixture (UNG), Lyophilized-After re dissolution of cillin bottles	12.5μL	
Primer probe mix[1)]	XμL	
Template DNA[2)]	5μL	
50×ROX reference dye(optional)[3)]	0.5μL	1x
ddH_2O	Up to 25μL	

Note:1)Typically, the final concentration of the primer is 0.2μM can achieve good results, ranging from 0.1 to 1.0μM serves as a reference for setting the range.The final concentration of the probe used is related to the fluorescent quantitative PCR instrument used, the type of probe, and the type of fluorescent labeling substance. Please refer to the instrument manual for actual use,Or adjust the concentration according to the specific usage requirements of each fluorescent probe.

2)The amount of DNA template used in the table is 5μ.For example, the amount of template and redissolved water can be adjusted by oneself.

3)The excitation optical systems of different instruments vary, and 50% can be added according to the instrument used for fluorescence quantification×Low ROX or 50×High ROX. Please refer to the table below for the usage of ROX reference dies for different models.

Instruments without adding ROX correction	Roche LightCycler 480，Roche LightCyler 96，Bio-rad iCyler iQ，iQ5，CFX96
Instruments that require Low ROX calibration	ABI Prism7500/7500 Fast，QuantStudio® 3 System，QuantStudio® 5 System QuantStudio® 6 Flex System，QuantStudio® 7 Flex System，ViiA 7 system，Stratagene Mx3000/Mx3005P，Corbett Rotor Gene 3000
Instruments requiring High ROX correction	ABI Prism7000/7300/7700/7900，Eppendorf，ABI Step One/Step One Plus

PCR reaction conditions

Procedure	Temperature	Time	Cycles
UNG digestion	50°C	2min	1
Pre denaturation	95°C	30s[1)]	1
Denaturation	95°C	10s	45
Annealing/Extension	60°C	20s[2)]	

Note:1)The enzyme used in this product is activated under pre denaturation conditions of 95℃and 30seconds. Under this condition, most templates can perform well in de chaining.For templates with high GC content and complex secondary structures, the pre denaturation time can be extended to 1minute to fully unwind the initial template. If the high-temperature treatment time is too long, it will affect the enzyme activity; For simple templates, pre denaturation for 20seconds can also be used, and the optimal pre denaturation time can be determined based on the template situation.

2)It is recommended to use a two-step PCR reaction program, and the annealing temperature should be set at 58-64℃as a reference range. When non-specific reactions occur, the annealing temperature can be increased.If good experimental results cannot be obtained due to the use of primers with lower Tm values or excessively long amplification products, a three-step PCR amplification can be attempted. The annealing temperature should be set within the range of 56℃-64℃ as a reference.

The annealing extension time settings for several common instruments are as follows:

1. When using Roche, BioRad, Agilent, and companies such as Hongshi and Dongsheng for fluorescence quantitative PCR, please set it at 20 seconds.
2. When using ABI 7000/7300/7500, please set it to 30 seconds.
3. The annealing and extension time can be set according to the use of different models of instruments and templates. Please follow the requirements of the instrument user manual for experimental operations.