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Animal Detection Probe Mixture, UNG (Mg^{2+} free) Lyophilized

Product Number:PCM22

Shipping and Storage

-20±5˚C. Short term use can be stored in 2-8˚C. Try to avoid repeated freeze-thaw cycles as much as possible.

Components

Component	PCM21 48rxns	PCM21 100rxns
2×Animal Detection Probe Mixture, UNG (Mg^{2+} free) Lyophilized	1ml	5ml
AD freeze-drying protective agent	500μl	2.5ml

Description

The 2×Animal Detection Probe Mixture, UNG (Mg^{2+} free) Lyophilized is a specialized freezeable intervention mixture that does not contain Mg^{2+} and is suitable for probe detection of DNA viruses, with a concentration of 2×,contains novel antibody modified Taq DNA polymerase, PCR buffer, dNTPs, as well as enhancers and stabilizers, making it convenient and fast to use. This product does not contain Mg^{2+}, and the Mg^{2+}content can be adjusted to meet different amplification needs.This product is compatible with single and multiple probe qPCR reaction systems, and can also be used for the preparation of freeze-drying detection reagents.

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.

Preparation and precautions before use

1. Before use, please gently mix the product upside down after it has completely melted, avoiding foaming as much as possible, and use it after a brief centrifugation.
2. AD freeze-drying protective agent can be melted in a 70˚C water bath for later use.
3. This product can be stored for a long time at -20˚C. If frequent use is required in the short term, it can be used between 2-8˚C save.
4. ROX dye is used to correct the fluorescence signal error generated between quantitative PCR wells, and this product does not contain ROX dye

Protocol

1. PCR reaction system

Reagent	50μL PCR reaction	25μL PCR reaction	Final Concentration
2×Animal Detection Probe Mixture, UNG (Mg^{2+} free) Lyophilized	25μL	12.5μL	1×
AD freeze-drying protective agent	12.5μL	6.25μL	
Mg^{2+}	As required	As required	
Forward Primer，10μM	1μL	0.5μL	0.2μM[1)]
Reverse Primer，10μM	1μL	0.5μL	0.2μM
Probe[2)]	1μL	0.5μL	0.2μM
Template DNA[3)]	XμL	XμL	
50×ROX reference dye(optional)[4)]	1μL	0.5μL	1×
ddH_2O	Up to 50μL	Up to 25μL	

Note:1)Usually, a primer final concentration of 0.2μM can yield good results, which can be used as a reference for setting the range from 0.1-1.0μM. In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific reactions occur, the concentration of primers can be reduced to optimize the reaction system.

2)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 or the specific usage requirements of each fluorescent probe for concentration adjustment during actual use.

3)Usually, the amount of DNA templates is based on 10-100 ng genomic DNA or 1-10 ng cDNA. Due to the different copy numbers of target genes contained in templates of different species, gradient dilution can be performed on the templates to determine the optimal template usage.

4)The excitation optical systems of different instruments vary,Choose to add 50×Low ROX or 50×High ROX based on the instrument used for fluorescence quantification.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

2. PCR reaction procedure

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:

When using Roche, BioRad, Agilent, and companies such as Hongshi and Dongsheng for fluorescence quantitative PCR, please set it at 20 seconds.

When using ABI 7000/7300/7500, please set it to 30 seconds.

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.

3. Freeze drying procedure

Stage	Step	Temperature	Slope time	Temperature control time	vacuum degree Pa	notes

precool	1	0˚C		30 min	-	The fastest cooling rate
Pre freezing	2	-50˚C	-	3 h	-	at room temperature
	3	-30˚C	-	3 h	14	can be set with a slope
Sublimation drying	4	-10˚C	-	2 h	14	time, and slope control
	5	0˚C	-	1 h	14	can be used to increase
desorption drying	6	30˚C	-	4 h	14	temperature

Note:1)Minor changes in the ingredient formula require re determination of freeze-drying parameters and corresponding adjustments.

2)Requirements for freeze-drying equipment: surface temperature of cold trap coils≤-50℃.

Plate layer temperature≤-45℃. Temperature uniformity±1℃.

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

3)Environmental requirements: Solution packaging and configuration should be carried out under 10000 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.