Version: 02/2024

Lyophilized Animal Detection Probe Mixture (UNG)

Cat. No. : CW3221S (48 rxns).
CW3221M（100 rxns）

Storage Condition: 4-30 °C storage. After redissolution, it can be stored at -20 °C. Avoid repeated freeze-thaw.

Components

Component	CW3221S 48 rxns	CW3221M 100 rxns
Lyophilized Animal Detection Probe Mixture（UNG）	6×8-strip tubes	1×penicillin bottle

Introduction

Lyophilized Animal Detection Probe Mixture (UNG) is a dedicated in situ full-component lyophilized reagent for probe-based detection of DNA viruses, including new antibody-modified Taq DNA polymerases, PCR buffers, dNTPs, Mg^{2+}, enhancers and stabilizers. It is only needed to add the extracted template, primers, probes, which is easy to use. The product is compatible with both single and multiplex probe method qPCR reactions.

The dUTP-UNG anti-contamination system is used in this product. dUTP is added during the preparation of the PCR reaction system, therefore form the amplification product containing dU bases. This product can be eliminated by the UNG enzyme treatment in the PCR system before the next PCR reaction. This effectively removes residual contamination of the PCR product and greatly reduces false positives due to contamination of the amplification product. The UNG enzyme is inactivated by the pre-denaturing step in the PCR cycle, so it does not affect the formation of new dU-based PCR products.

Note:

1) This product can be activated at 95 °C for 30 s. For complex templates, pre-denaturation time can be extended to 1 min. For simple templates, pre-denaturation time can also be set for 20 s. The optimal pre-denaturation time can be determined according to the template situation.

2) It is recommended to use two-step PCR reaction procedure. The reference range of 58-64 °C can be used. If the specificity of the reaction should be increased, the annealing temperature can be increased. If good experimental results are not obtained due to the use of primers with low Tm values, three-step PCR amplification can be attempted, and the reference range of 56-64 °C can be used.

The annealing/extension time is set as follows for several common instruments:

When using Roche, BioRad, Agilent, Hongshi, Eastwin and other companies, please set it to 20 s.

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

The annealing and extension time can be set according to the use of different types of instruments and different templates, please follow the requirements of the instrument instruction manual for experimental operation.

Precautions

1. This product can be stored at 4-30 ℃ for long-term storage and -20 ℃ for longer periods of time. If it is not used up after redissolution, 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. This product does not contain ROX dye, if the instrument used needs to match ROX dye, please contact the local salesman or Cowin's customer service.

PCR Reaction System for 8-Strip Tubes

Reagent	25 μL System	Final Concentration
Lyophilized Animal Detection Probe Mixture（UNG）	1 well	
ddH_2O	12.5 μL	
Primer Probe Mix[1)]	X μL	
Template DNA[2)]	5 μL	
50×ROX reference dye (optional)[3)]	0.5 μL	1×
ddH_2O	to 25 μL	

PCR Reaction System for Penicillin Bottle

Add 1.3 mL of ddH_2O to the penicillin bottle to redissolve, vortex and mix for 10 s, and briefly centrifuge for later use.

Reagent	25 μL System	Final Concentration
Lyophilized Animal Detection Probe Mixture（UNG）- after redissolution	12.5 μL	
Primer Probe Mix[1)]	X μL	
Template DNA[2)]	5 μL	
50×ROX reference dye (optional) [3)]	0.5 μL	1×
ddH_2O	to 25 μL	

Note:

1) Generally, a better result can be obtained with a primer concentration of 0.2 μM. 0.1-1.0 μM can be set as a reference. The concentration of the probe used is related to the type of real-time PCR instruments, probe types, and fluorescent label substances used. Please refer to the instrument manual or the specific use requirements of each fluorescent probe for concentration adjustment.

2) The DNA template quantity is illustrated as 5 μL in the table above. The amount of template and reconstitution water can be adjusted accordingly.

3) The excitation optical system of different instruments is different, and 50×Low ROX or 50× High ROX can be added according to the instrument using fluorescence quantification.

Instruments that do not require ROX calibration	Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96, etc.
Instruments requiring 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, etc.
Instruments requiring High ROX calibration	ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus, etc.

PCR Reaction Conditions

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