PrimeTaq™ Probe qPCR MasterMix (with HL-UDG)

User's Instruction

Description

PrimeTaq™ Probe qPCR MasterMix (with HL-UDG) has an extremely strong recognition ability for dUTP, and can achieve optimal anti contamination ability with Heat-Labile Uracil DNA Glycosylase (HL-UDG), which can be irreversibly inactivated by heat denaturation at 95°C for 5 min, without affecting subsequent PCR reactions. PrimeTaq™ Probe qPCR MasterMix (with HL-UDG) is a premix composed of chemically modified PrimeTaq™ HotStart Direct PCR DNA Polymerase, reaction buffer, dNTPs, UDG, etc., in which the dTTP is completely replaced by dUTP, so that all the amplified products contain dUTP, and the CT can be postponed by more than 15 × (> 99.997% contamination removal) under test conditions of 105 copies of the contaminant. Therefore, PrimeTaq™ Probe qPCR MasterMix (with HL-UDG) has excellent performance against nucleic acid aerosol contamination.

This MasterMix does not contain ROX dyes, which is compatible with a wide range of fluorescent probes and a variety of quantitative PCR instruments. In addition, this MasterMix has great performance in multiplex quantitative PCR (multiplex qPCR) assays, enabling a minimum of 4-plex quantitative PCR assays.

PrimeTaq™ HotStart Direct PCR DNA Polymerase in this MasterMix has high impurity tolerance and it is extremely resistant to ethanol, guanidinium salts, heparin, serum, plant polysaccharide polyphenols. Therefore, it can be used for direct quantitative PCR detection of crude samples. With hot start property, the polymerase is 100% inactive below 50°C and can be completely recovered only after heating at 95°C for 5 min. Therefore, this system can effectively inhibit nonspecific PCR amplification, greatly improving the specificity of PCR amplification.

Kit Contents

	100 preps
2 × PrimeTaq™ Probe qPCR MasterMix (with HL-UDG)	1 ml

Tolerance to inhibitors

Impurity	Amount	Impurity	Amount
SDS	0.01%	Guanidine Salt	0.25%
Ethanol	5%	Whole Blood	15%
Heparin	0.1 IU/ml	Serum	15%
Trizol	0.50%	Plasma	2%
Hemoglobin	30μm	Urine	5%

Protocol

Set up qPCR reaction

1. Set up qPCR reaction as the following table (take 20 μl per well as an example):

Reagent	Volume	Final Concentration
2 × PrimeTaq™ Probe qPCR MasterMix (with HL-UDG)	10 μl	1×
Primer F (20 μM)	0.1-0.4 μl	0.1-0.4 μM
Primer R (20 μM)	0.1-0.4 μl	0.1-0.4 μM
Probe	0.1-0.4 μl	0.1-0.4 μM
Template DNA	0.5-2 μl	
ddH_2O	Up to 20 μl	-

Thermocycling Conditions for qPCR (Two-Step, recommended)

1. Decontamination: 25°C for 2 min
    a) Note: the temperature setting in this step can be varied from 25-37°C without significant difference, but temperatures above 42°C can cause a dramatic decrease in the digestion capacity of UDG.
2. Hot Start: 95°C for 5 min
3. 40 cycles:
    a) 95°C for 5 sec

b) 55-65°C for 30 sec (collect signal)

Thermocycling Conditions for qPCR (Three-Step, recommended)

In most cases, ideal amplification can be achieved using two-step procedure. In case where the expected results cannot be achieved, a three-step procedure can also be used.

1. Decontamination: 25°C for 2 min
    a) Note: the temperature setting in this step can be varied from 25-37°C without significant difference, but temperatures above 42°C can cause a dramatic decrease in the digestion capacity of UDG.
2. Hot Start: 95°C for 5 min
3. 40 cycles:
    a) 95°C for 5 sec
    b) 55°C for 10 sec
    c) 72°C for 30 sec

Storage

Minimum shelf life is 2 years under -20°C with light-free.