HB220429

Hieff Unicon™ qPCR TaqMan Probe Master Mix

Product Information

Product Name	Cat#	Size
Hieff Unicon™ qPCR TaqMan Probe Master Mix	11205ES03	1 mL
	11205ES08	5 mL
	11205ES25	25 mL

Product Description

The product is a premixed solution for 2× real-time quantitative PCR amplification by the probe method. The antibody hot-start UNICON™ DNA Polymerase (Cat#10113) used can effectively inhibit the amplification caused by the non-specific annealing of primers. At the same time, the formula optimizes the factors that can effectively inhibit non-specific PCR amplification and improve the amplification efficiency of PCR reaction, which is suitable for the amplification of low-concentration templates, so that quantitative PCR can obtain a good standard curve in a wide quantitative area.

Product Components

Component Name	Components	Cat#/Size		
		11205ES03 (1 mL)	11205ES08 (5 mL)	11205ES25 (25 mL)
11205-A	Hieff Unicon™ qPCR TaqMan Probe Master Mix	1 mL	5 × 1 mL	25 mL
11205-B	50×Low Rox	40 μL	200 μL	1 mL
11205-C	50×High Rox	40 μL	200 μL	1 mL

Shipping and Storage

The product is shipped with ice packs and can be stored at -20°C away from light for 18 months. Please avoid repeated freeze-thaw.

Reaction System

Components	Volume	Volume	Final Concentration
Hieff Unicon™ qPCR TaqMan Probe Master Mix	10 μL	25 μL	1×
Forward Primer (10 μmol/L)	0.4 μL	1 μL	0.2 μmol/L
Reverse Primer (10 μmol/L)	0.4 μL	1 μL	0.2 μmol/L
TaqMan Probe (10 μmol/L)	0.2 μL	0.5 μL	0.1 μmol/L
50×High or Low Rox	0.4 μL	1 μL	1×
Sample DNA	X μL	X μL	-
Sterile ultrapure H_2O	to 20 μL	to 50 μL	-

[Notes]: Be sure to mix well before use to avoid excessive air bubbles caused by vigorous shaking.

1) Reference Dye: The addition of Rox can be selected according to different instrument models. For details, please refer to [Applicable Models];

2) Primer Concentration: Usually, the final primer concentration is 0.2 μmol/L, and it can also be adjusted between 0.1-1.0 μmol/L according to the situation;

3) Probe Concentration: final probe concentration between 50 nmol/L-250 nmol/L;

4) Template Concentration: If the template type is undiluted cDNA stock solution, the volume used should not exceed 1/10 of the total volume of the qPCR reaction;

5) Template Dilution: 5-10 times dilution of the cDNA stock solution is recommended, and the optimal amount of template added is 20-30 cycles for the CT

value obtained by amplification;

6) Reaction System: 20 μL or 50 μL is recommended to ensure the validity and repeatability of target gene amplification;

7) System Preparation: Please prepare in an ultra-clean workbench, and use pipette tips and reaction tubes without nuclease residues; it is recommended to use pipette tips with filter elements. Avoid cross-contamination and aerosol contamination;

Amplification Procedure(Two-Step Procedure)

Stage	Temperature	Time	Cycles
Reverse Transcription	95℃	1 min	1
Transcription	95℃	10 sec	40
Annealing and Elongation	60℃	30 sec	

[Notes]:

a) Pre-denaturation time: According to the specific conditions of different templates and primers, the pre-denaturation time can be appropriately increased to 3-5 min;

b) Annealing temperature and time: It is recommended to adjust within the range of 56-64°C. If the reaction effect is not good, the reaction time can be appropriately extended;

c) Fluorescence signal acquisition: Please refer to the instrument settingst;

Result Analysis

Quantitative experiments require at least three biological replicates.

After the reaction is complete, the amplification curve needs to be confirmed. When performing PCR quantification, it is necessary to create a standard curve.

Reaction specificity requires confirmation by agarose gel electrophoresis.

Primer Design Guidelines

1) The recommended primer length is about 25 bp. The length of the amplified product is preferably 150 bp, not less than 100 bp, and can be selected within 100 bp-300 bp.

2) The difference between the Tm values of the forward primer and the reverse primer should not exceed 2°C.

3) The base distribution of primers should be uniform, avoid four consecutive identical bases, and the GC content should be controlled at about 50%. The last base at the 3' end is preferably G or C.

4) It is best to avoid complementary sequences of more than 3 bases within the primers or between the forward and reverse primers.

5) Primer specificity needs to be checked with the NCBI BLAST program. Avoid non-specific complements of more than 2 bases at the 3' end of the primers.

TaqMan Probe Design Guidelines

1) The length of the probe is generally 18-40 bp. The probe sequence should be as close as possible to the forward or reverse primer, but should not have overlapping regions with it.

2) The annealing temperature of the probe should be 65-67℃.

3) The base distribution of primers should be uniform, and 4 consecutive identical bases should be avoided. The base G should be avoided at the 5' end of the probe.

4) If the sequence contains a polymorphic site, it should be located in the middle of the probe sequence.

Applicable Models

ABI Series: ABI 5700, 7000, 7300, 7700, 7900HT Fast, StepOne, StepOne Plus; 7500, 7500 Fast, ViiA7, QuantStudio 3 and 5, QuantStudio 6, 7, 12k Flex;

Bio-Rad Series: Bio-Rad CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4;

Eppendorf Mastercycler ep realplex, realplex 2 s;

Qiagen Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000;

Roche Series: Roche Applied Science LightCycler 480, LightCycler 2.0; Lightcycle r 96;

Thermo Scientific PikoReal Cycler;

Cepheid SmartCycler; **Illumina** Eco qPCR.