



Ver. HB230112

# Hieff Unicon™ qPCR TaqMan Probe Master Mix

## 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 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.

## Components

Components No.	Name	11205ES03	11205ES08	11205ES25
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

## Specifications

Hot Start	Built-in hot start
Detection method	Primer-probe detection
PCR method	RT-qPCR
Polymerase	Taq DNA polymerase
Type of sample	RNA
Application equipment	Applied Biosystems: 5700, 7000, 7300, 7700, 7900HT Fast, StepOne™, StepOne™ Plus; 7500, 7500 Fast, ViiA™7, QuantStudio™ 3 and 5, QuantStudio™ 6, 7, 12k Flex; 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 Applied Science: LightCycler 480, LightCycler 2.0; Lightcycle r 96 Thermo Scientific: PikoReal Cyler; Cepheid: SmartCyler; Illumina: Eco qPCR

## Storage

The product should be stored at -25°C~-15°C away from light for 18 months. Please avoid repeated freeze-thaw.



## Instructions

### 1. Reaction System

Components	Volume (μL)	Volume (μL)	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 <sub>2</sub> O	to 20 μL	to 50 μL	-

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

1.1 Reference Dye: The addition of Rox can be selected according to different instrument models.

1.2 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.

1.3 Probe Concentration: final probe concentration between 50 nmol/L-250 nmol/L.

1.4 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.

1.5 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.

1.6 Reaction System: 20 μL or 50 μL is recommended to ensure the validity and repeatability of target gene amplification.

1.7 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.

### 2. Reaction program

Stage	Temperature	Time	Cycles
Reverse Transcription	95°C	1 min	1
Transcription	95°C	10 s	40
Annealing and Elongation	60°C	30 s	

[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 settings.

### 3. 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.

### 4. Primer design guidelines



- 4.1 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.
- 4.2 The difference between the  $T_m$  values of the forward primer and the reverse primer should not exceed 2°C.
- 4.3 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.
- 4.4 It is best to avoid complementary sequences of more than 3 bases within the primers or between the forward and reverse primers.
- 4.5 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.

#### **5. TaqMan probe design guidelines**

- 5.1 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.
- 5.2 The annealing temperature of the probe should be 65-67°C.
- 5.3 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.
- 5.4 If the sequence contains a polymorphic site, it should be located in the middle of the probe sequence.

#### **Notes**

1. Dissolve at room temperature, store in ice box or on an ice bath after dissolution, and store at -20°C immediately after use.
2. For your safety and health, please wear lab coats and disposable gloves for operation.
3. This product is for research use ONLY!