Servicebio®2×Multiplex Probe qPCR Master Mix with UDG (None ROX)

Cat. #：G3347

Product Information

Product Name	Cat.No.	Spec.
2×Multiplex Probe qPCR Master Mix with UDG (None ROX)	G3347-01	1 mL
	G3347-05	5×1 mL
	G3347-15	15×1 mL

Description/Introduction

The product is a 2×fluorescence quantitative PCR premixture (None ROX) based on probe method, it contains hot-start TaqDNA polymerase, dNTP and necessary buffer components, and can simultaneously perform up to quadruple fluorescence quantitative PCR reaction in the same reaction tube. The premix does not contain correction dyes and is suitable for equipment without ROX correction. The addition of blue non-fluorescent dye to this product can avoid sampling errors. In addition, the product also includes Uracil-DNAGlycosylase (UDG) and specially optimized dUTP addition amount, which can prevent template residual contamination and improve the specificity, sensitivity and accuracy of the product.

Storage and Handling Conditions

Shipped with wet ice and stored at -20°C; valid for up to 12 months.

Product Contents

Component Number	Component	G3347-01	G3347-05	G3347-15
G3347-01	2×Multiplex Probe qPCR Master Mix with UDG (None ROX)	1 mL	5×1 mL	15×1 mL
Manual		One copy		

Before starting (please read carefully)

1. Real-time fluorescence quantitative PCR instrument is required.
2. Special qPCR reaction tubes or reaction plate for experiment are required but not supplied in this kit.
3. QPCR primers and probes (reference primers and probe design principles) are required but not supplied in this kit.

Assay Protocol / Procedures

1. Recommend PCR reaction system:

Component	20 μL rxn	Final Concentration
2×Multiplex Probe qPCR Master Mix with UDG (None ROX)	10 μL	1×
Forward Primer (10 μM)[a]	Variable	0.1-0.5 μM
Reverse Primer (10 μM)[a]	Variable	0.1-0.5 μM
Probe (10 μM)[a]	Variable	0.1-0.5 μM
Template[b]	Variable	as required
Nuclease-free Water	Add to 20 μL	

a. Usually, a good amplification effect can be obtained with the final concentration of 0.2 μM. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.2-1.0 μM.

b. The amount of template addition varies with the number of copies of target genes in the template solution. Gradient

Wuhan Servicebio Technology Co.,Ltd.
Add: 5th Floor,22 building, Biopark, Gaoxin Second Road 388, East Lake High-Tech Developing Zone,Wuhan,China 430000
Tel: 027-5111-3188 Website: www.servicebio.com

dilute the template and investigate the appropriate amount of template addition. In the 20 μL reaction system, the amount of template DNA should be less than 100 ng. When using cDNA (RT reaction solution) of RT-PCR reaction as template, the addition amount should not exceed 10% of the total volume of PCR reaction solution.

2. PCR reaction procedure (can be adjusted according to the model):

A. Two-step method					B. Three-step method				
Stage	**Step**	**Cycle**	**Temp**	**Time**	**Stage**	**Step**	**Cycle**	**Temp**	**Time**
Stage 1	UDG incubation	1	50°C	2 min	Stage 1	UDG incubation	1	50°C	2 min
Stage 2	Pre-denaturation	1	95°C	30 sec	Stage 2	Pre-denaturation	1	95°C	30 sec
Stage 3	Denaturation	40	95°C	15 sec	Stage 3	Denaturation	40	95°C	15 sec
	Annealing /Extension		60°C	30 sec[a]		Annealing		55-65 °C	10 sec
						Extension		72°C	30 sec[a]

a. To improve the specificity of amplification, the two-step procedure can be used or the annealing temperature can be increased. To improve the amplification efficiency, the three-step procedure can be used or the extension time can be extended.

Note

1. After thawing, please gently mix up and down, do not vortex, avoid bubbles, mix well before use.
2. When preparing the reaction solution, please place the reagent on the ice.
3. The product contains fluorescent dyes, so strong light should be avoided when preparing qPCR reaction solution.
4. New disposable tips should be used for preparation of reaction mixes to avoid cross contamination.
5. Avoid freeze-thawing cycles of the Master Mix, and try to use it up within a month after thawing.

Compatible Instruments

ABI: PikoRealTM Cycler;

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

Eppendorf: Realplex 2s, Mastercycler® ep, realplex;

Illumina: Eco QPCR;

Cepheid: SmartCycler®;

Qiagen Corbett: Rotor-Gene® series;

Roche: LightCycler™ series;

Takara: Thermal Cycler Dice series;

Analytikjena: qTOWER series;

Hangzhou Bori: LineGene series;

Primer Design Principles

Taqman primer design principle

1. Determine the probe before designing primers.
2. When designing primers, get as close to the probe as possible without overlapping the probe.
3. Avoid using 4 or more consecutive G.
4. The Tm value of each primer should be 58-60°C.
5. The last 5 nucleotides at the end of the primer cannot have more than 2 G and C.
6. Primers had better not contain self-complementary sequences, otherwise they will form the hairpins.
7. In order to avoid the amplification of the genome, it is best to design primers across exons.
8. The length of amplification product should be 50-150 bp in order to obtain the best PCR efficiency.
9. No other non-specific products were found in the comparison results on NCBI.

Wuhan Servicebio Technology Co.,Ltd.
Add: 5th Floor,22 building, Biopark, Gaoxin Second Road 388, East Lake High-Tech Developing Zone,Wuhan,China 430000
Tel: 027-5111-3188 Website: www.servicebio.com

Taqman probe design principle

1. Probe length should be 13-25 bp (13-30 bp if conventional TaqMan probe is used).
2. The Tm value should be 65°C~70°C, which is usually 5°C~10°C higher than the TM value of the primer to ensure that the probe preferentially binds to the target gene during annealing.
3. For a primer, the content of guanine-cytosine (G+C) should be between 40% and 70%.
4. The 5 'end of the probe should avoid using G, because the 5' end G will have quenching effect, even if it is cut off.
5. In the whole probe, the content of C is obviously higher than that of G, and the high content of G will have quenching effect, so we can choose another paired chain as the probe.

Taqman MGB probe design principle

1. A report dye (for example, FAM™) is attached to the 5 'end of the probe.
2. There is a non-fluorescence quenching group (NFQ) at the 3 'end of the probe.
3. The part of MGB is attached to NFQ, and MGBs increases the annealing temperature (Tm) without increasing the length of the probe, so a shorter probe can be designed, but not less than 13 bp.
4. In principle, as long as there is a base mutation in the MGB probe, MGB can detect it (the MGB probe will not bind to the target gene and will not produce a fluorescent signal).

For Research Use Only!

Version number：V1.1-202302

Wuhan Servicebio Technology Co.,Ltd.
Add: 5th Floor,22 building, Biopark, Gaoxin Second Road 388, East Lake High-Tech Developing Zone,Wuhan,China 430000
Tel: 027-5111-3188 Website: www.servicebio.com