Taq Pro HighGC Multiple Probe qPCR Mix

QN211-EN

Vazyme

Instruction for Use

Version 22.1

Vazyme

Contents

01/Product Description

Taq Pro HighGC Multiple Probe qPCR Mix is a master mix for probe qPCR to detect DNA templates (such as DNA viruses). The core component, Taq Pro HS DNA Polymerase, is a new generation hot-start DNA polymerase that has been modified based on antibody techniques and upgraded to improve template affinity. Equipped with the most suitable buffer optimized for the qPCR system, amplification specificity and sensitivity for detection of low-copy genes are significantly improved. It can provide excellent amplification curve within a wide quantitative range, and accurately quantify and detect target genes, with good repeatability and high reliability. This product is suitable for the amplification of rich GC templates and has good impurity tolerance. The product is a 2 × master mix. Only primers, probes and templates need to be added additionally. It is convenient to use and compatible with fast program to reduce test time.

02/Components

Components	QN211-EN01 (100 rxns/ 20 µl reaction)	QN211-EN02 (500 rxns/ 20 µl reaction)	QN211-EN03 (2,500 rxns/ 20 µl reaction)
2 × Taq Pro HS High GC Multiple Probe qPCR Mix[a]	1 ml	5 × 1 ml	
50 × ROX Reference Dye 1[b]	100 µl	200 µl	5 × QN211-EN02
50 × ROX Reference Dye 2[b]	100 µl	200 µl	

a. It contains dNTP Mix, Mg^{2+}, Taq Pro HS DNA Polymerase, etc.

b. It is used to correct the error of fluorescence signals between wells. Use 50 × ROX Reference Dye 1 for ABI 7900HT/7300 Real-Time PCR System and StepOnePlus; Use 50 × ROX Reference Dye 2 for ABI 7500, 7500 Fast Real-Time PCR System, and Stratagene Mx3000P. Don't use ROX for Roche and Bio-Rad Real-Time PCR instruments.

03/Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

04/Applications

This product is suitable for probe-based qPCR detection of animal, plant and microbial DNA.

05/Notes

For research use only. Not for use in diagnostic procedures.

Primer Design Guidance

1. The ideal primer length is 17 - 25 bp. Primers that are too short are likely to result in reduced amplification efficiency. Primers that are too long are more likely to generate higher structures. Both of them will interfere with the accuracy of the quantitative results.
2. Control the GC content of the primers at 40% - 60%, and the optimum GC content is from 45% - 55%.
3. The Tm value of the primer should be greater than 60℃. Primer Premier 5 is recommended to calculate the Tm value.
4. The overall distribution of A, G, C, and T in the primer should be as even as possible. Avoid using regions with high GC or TA contents, especially at the 3' end. Regions with uneven GC content must be avoided.
5. Try to avoid structures with consecutive T/C or A/G when designing primers.
6. The last five bases at the 3' end of the primer must not contain more than two G or C.
7. Ensure that the forward or the reverse primer is as close as possible to the probe sequence, but does not have overlap regions with the probe sequence.

TaqMan Probe Design Guidance

1. The probe sequence should be as close as possible to the forward or reverse primer, but overlap sequences must be avoided.
2. The probe length is typically 18 - 40 bp.
3. Avoid consecutive identical bases, especially four or more consecutive G.
4. Avoid using G at the 5' end of the probe.
5. The annealing temperature of the probe should be 65 ~ 67℃.
6. If there is polymorphic locus in the sequence, make it lie in the middle of the probe.

06/Experiment Process (Take ABI 7500 as the test instrument)

1. Prepare the following mixture in a qPCR tube:

2 × Taq Pro HighGC Multiple Probe qPCR Mix	10 μl
Primer Mix	X μl
TaqMan Probe Mix	Y μl
50 × ROX Reference Dye 2	0.4 μl
Template DNA	Z μl
ddH_2O	Up to 20 μl

The volume of each component in the reaction system can be adjusted according to the following principles:

▲ Generally, the final concentration of primer in the reaction system is 0.2 μM to obtain better amplification effect. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.1 - 1.0 μM.

▲ The final concentration of TaqMan Probe can be adjusted between 50 - 250 nM.

▲ Due to the high sensitivity of qPCR, the accuracy of template volume has a significant impact on qPCR results. In order to effectively improve the repeatability of the experiment, it is recommended to dilute the template and add it to the reaction system.

▲ Template can be adjusted to an appropriate volume. For example, the volume of undiluted cDNA template should be ≤1/10 of total volume.

2. Run the qPCR program as follows:

Standard Program

Stage 1	Contamination Digestion	Rep: 1	37˚C	2 min
Stage 2	Initial Denaturation	Rep: 1	95˚C	30 sec
Stage 3	Cycles	Reps: 45	95˚C	10 sec
			60˚C	30 sec

Fast Program

Stage 1	Contamination Digestion	Rep: 1	37˚C	2 min
Stage 2	Initial Denaturation	Rep: 1	95˚C	20 sec
Stage 3	Cycles	Reps: 45	95˚C	1 sec
			60˚C	20 sec*

* Please conduct preliminary experiments for the first attempt to confirm whether the fast program is compatible with the qPCR instrument.

07/FAQ & Troubleshooting

◇ Abnormal shape of amplification plot

① Rough amplification plot: The signal is too weak and generated after system correction. Increase template concentration and retry.

② Broken or downward amplification plot: The template concentration is too high and the baseline endpoint value is greater than C_T value. Reduce the baseline endpoint (C_T value - 4) and repeat data analysis.

③ Amplification plot goes downward suddenly: There are bubbles remaining in the reaction tube. Pay attention to centrifugation when processing samples and carefully check the reaction tube for any remaining bubbles before performing reaction.

◇ No amplification plot

① Insufficient number of reaction cycles: In general, the number of cycles is set to 45, but it should be noted that too many cycles will increase too many background signals and reduce the reliability of the data.

② Confirm whether the signal acquisition step is set up in the program: The two-step amplification program generally sets the signal acquisition at the annealing and extension stage, while the three-step amplification program should set the signal acquisition at the 72℃ extension stage.

③ Confirm whether the primers are degraded: Primers that have not been used for a long time should be tested for integrity using PAGE electrophoresis before use in order to rule out the possibility of degradation.

④ Low template concentration: Reduce the dilution factor and repeat the test. In general, samples with unknown concentration should be started at the highest concentration.

⑤ Template degradation: Prepare new template and retry.

◇ C_T value appears too late

① Low amplification efficiency: Optimize the reaction system, try the three-step amplification program or redesign the synthetic primers.

② Low template concentration: Reduce the dilution factor and repeat the test. In general, samples with unknown concentration should be started at the highest concentration.

③ Template degradation: Prepare new template and retry.

④ Long PCR products: The recommended length of PCR product is 80 - 150 bp.

⑤ PCR inhibitors in the system: They are usually introduced along with the template. Increase the dilution factor or prepare new template and retry.

◇ Amplification observed in negative control

① Contaminated of reaction system: Replace with new enzymes, Buffer, ddH_2O, primers, and probes, and retry.

◇ Standard curve linearity is poor for absolute quantification

① Sample loading error: Increase the template dilution factor and the loading volume of sample.

② Standard product degradation: Prepare the standard again and repeat the test.

③ Template concentration is too high: Increase the template dilution factor.

◇ Poor experiment repeatability

① Inaccurate pipetting volume: Use higher performance pipette; increase the template dilution factor, and increase the sample loading volume.

② Differences in temperature control between wells in qPCR instrument: Calibrate the instrument regularly.

③ Low template concentration: The lower the template concentration, the worse the repeatability. Reduce the template dilution factor or increase the volume of sample addition.

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