BioSmart U+ All-Powerful Multiple Probe qPCR PreMix (ONE TUBE)

QN222

Versioin 24.2

Product Description

BioSmart U+ All-Powerful Multiple Probe qPCR PreMix (ONE TUBE) is a probe-based qPCR premix for DNA multiplex amplification, which supports premixing of primers and probes. The BioSmart DNA Polymerase is screened by Vazyme's unique BioSmart platform for enzymes with long sequence of shortest extended bases and strong 3' end mismatch recognition ability. With the high blocking rate dual-species specific antibody (binding to different antigenic epitopes) and an optimized buffer system, it effectively improves multiplex amplification sensitivity, specificity, low-copy detection rate, GC content compatibility and impurity tolerance (blood, ethanol, guanidinium salts, etc.). It supports fast program and primers and probes premixing. In addition, the dUTP/UDG anti-contamination system in 2 × All-Powerful qPCR PreMix effectively prevents aerosol contamination, ensuring accurate qPCR results.

Components

Components	QN222-01 200 rxns (20 μl/rxn)	QN222-02 500 rxns (20 μl/rxn)	QN222-03 2,500 rxns (20 μl/rxn)
2 × All-Powerful qPCR PreMix[a]	2 × 1 ml	5 × 1 ml	1 × 25 ml

a. It contains dNTP/dUTP Mix, Mg^{2+}, BioSmart DNA Polymerase, Heat-labile UDG, etc.

Storage

Store at -30 ~ -15°C and ship at ≤0°C.

Applications

It is applicable for multiplex quantification of DNA from all species sources, and the sample type can be genomic DNA, plasmid DNA, etc.

Applicable Instruments

It is applicable to, but not limited to, the following instruments: ABI QuantStudio 3, ABI QuantStudio 5, ABI 7500, etc.

Notes

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 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°C. 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 uniform as possible. Avoid using regions with high GC or TA content, 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 base G at the 5' end of the probe.
5. The annealing temperature of the probe should be 65 ~ 67°C.
6. If there is polymorphic locus in the sequence, make it lie in the middle of the probe.

Experiment Process (Using ABI QuantStudio 5 as a test machine)

1. Prepare the following mixture in a qPCR tube Components

Components	Volume
2 × All-Powerful qPCR PreMix	10 μl
Primer Mix	X μl
TaqMan Probe Mix	Y 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, a good result can be obtained when the final concentration of primer in the reaction system is 0.2 μM. If the result is not as expected, the primer concentration can be adjusted between 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 experiments, it is recommended to dilute the template and add it to the reaction system.

▲ The volume of templates can be adjusted appropriately.

▲ After the addition of samples, thoroughly mix the samples before conducting the test.

2. Perform qPCR reaction under the following conditions

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*

* For signal acquisition.

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 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. 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: Generally, set the signal acquisition at the annealing and extension stage in two-step amplification program, while set the signal acquisition at the 72°C extension stage in three-step amplification program.

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 Template concentration is too low: Reduce the dilution factor and repeat the experiment. Generally, start with the highest concentration for samples with unknown concentrations.

③ Template degradation: Prepare new template and retry.

④ PCR product is too long: Recommended PCR product length 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.

◇ Poor standard curve linearity for absolute quantification

① Reaction system contamination: Replace with new mix, ddH_2O and primers to repeat the experiment.

◇ 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 a precision 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.