# Taq Pro Universal SYBR qPCR Master Mix
# NB-54-0226-02
# NB-54-0226-03

Version 22.3

## Product Description

This product is a special premix for qPCR reaction using SYBR Green I chimeric fluorescence method. The core component, Taq Pro DNA Polymerase, is a new generation of hot-start polymerase modified by antibody method. It has many advantages such as strong specificity, high detection sensitivity and high amplification yield. With the combination of Buffer optimized for qPCR and specific enhancer, it is very suitable for qPCR reaction with high specificity and sensitivity. This kit contains Specific ROX Reference Dye, which is suitable for all qPCR instruments without adjusting the concentration of ROX on different instruments. This kit contains 2 × master mix that can be amplified by adding primers and template.

## Components

| Components | NB-54-0226-02 (500 rxns/20 µl reaction) | NB-54-0226-03 (2,500 rxns/20 µl reaction) |
|---|---|---|
| 2 × Taq Pro Universal SYBR qPCR Master Mix* | 4 × 1.25 ml | 5 × Q712-02 |

* It contains dNTP, $Mg^{2+}$, Taq Pro DNA Polymerase, SYBR Green I and Specific ROX Reference Dye, etc.

## Storage

Store at -30 ~ -15°C and protect from light. Master mix can be stored stably at 2 ~ 8°C for 6 months and protected from light after thawing. Transport at ≤ 0°C.

## Applications

It is applicable for amplification and quantification of DNA samples, which can amplify DNA from all species, including genomic DNA, cDNA, plasmid DNA, λDNA, etc.

## Notes

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

1. If white precipitate is found in the Master Mix after thawing, please place it at room temperature for a short while and invert it upside down several times to dissolve the precipitate before use.
2. Avoid repeated freezing and thawing, so as not to cause the decrease of enzyme activity. If the amount of each use is small, it is recommended to aliquot it to small portions.
3. Please invert the Master Mix upside down several times to mix thoroughly. Do not vortex to avoid air bubbles, which will affect the quantitative results. The Master Mix is ready to use after mixing and centrifuging briefly. Mix gently by pipetting. If the air bubbles are generated, please centrifuge again before use.
4. As this kit contains a fluorescent dye SYBR Green I, it should be stored protect from light. Avoid strong light when preparing the reaction solution.
5. Detection sensitivity of this kit is very high, easy to be contaminated by aerosols in the air. Therefore, the preparation of the reaction system should be carried out in clean bench. Sterile tips and reaction tubes should be used in the preparation process. If laboratory conditions permit, special pipettes and tips with filtering element are recommended.

## Experiment Process

**1. Prepare the following mixture in a qPCR tube**

| | |
|---|---|
| 2 × Taq Pro Universal SYBR qPCR Master Mix | 10.0 µl |
| Primer1 (10 µM) | 0.4 µl |
| Primer2 (10 µM) | 0.4 µl |
| Template DNA/cDNA | x µl |
| $ddH_2O$ | To 20.0 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.

▲ 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.

▲ The volume of undiluted cDNA template should be ≤1/10 of total volume.

▲ If white precipitate is found in the Master Mix after thawing, please place it at room temperature for a short while and invert it upside down several times to dissolve the precipitate before use.

**2. Perform the qPCR reaction according to the following conditions**

| | | | | |
|---|---|---|---|---|
| Stage 1 | Initial Denaturation[a] | Rep:1 | 95°C | 30 sec |
| Stage 2 | Cycling Reaction | Reps:40 | 95°C | 3 - 10 sec[b] |
| | | | 60°C | 10 - 30 sec[c] |
| Stage 3 | Melting Curved[d] | Rep:1 | 95°C | 15 sec |
| | | | 60°C | 60 sec |
| | | | 95°C | 15 sec |

a. Initial denaturation condition is suitable for most amplification reactions. If the template structure is complex, the initial denaturation time can be extended to 3 min to improve the initial denaturation effect.

b. For standard program, select 10 sec, and 3 sec can be selected for fast program.

c. Select 30 sec for standard program; Fast program: for amplicons within 200 bp, the shortest extension time can be set to 10 sec; for amplicons over 200 bp, the recommended extension time is 30 sec.

d. Different qPCR instrument types of melting curve acquisition procedures are not the same, and please select the instrument default melting curve acquisition procedures.

## 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 40, 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°C 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 PCR system, then try the three-step amplification program or redesign and synthesize 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 products 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 mix, dd$H_2O$ and primers to repeat the experiment. The reaction system should be prepared in clean bench to reduce aerosol contamination.

② Primer dimer: Carry out analysis in association with the melting curve.

◇ The linear relationship of standard curve is not satisfactory in absolute quantification

① Deviations of pipetting volume: Increase the dilution factor of template and increase the pipetting volume accordingly.

② Degradation of standards: Prepare new standards and retry.

③ High template concentration: Increase the dilution factor.

◇ Multiple peaks in the melting curve

① Inappropriate primer design: Design and synthesize new primers according to the primer design principles.

② High primer concentration: Decrease the primer concentration.

③ cDNA template with contamination of genomic DNA: Prepare new cDNA templates.

◇ 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.