# Operation Guide of SuperReal Color PreMix (Probe) (FP216)

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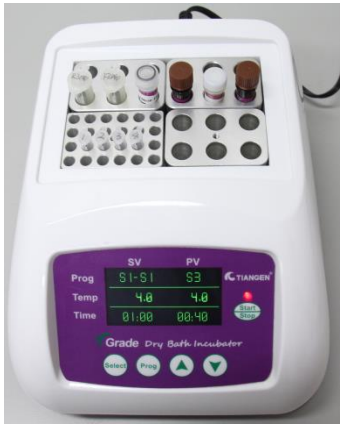
# Experiment Preparation

1. cDNA sample
2. Pipette and matching pipette tips (RNase-free)
3. 1.5 ml centrifuge tube (RNase-free) and 200 μl PCR tube (RNase-free)
4. Vortex oscillator, centrifuge and metal bath/thermal cycler

# Step 1






Thaw 2×SuperReal Color PreMix, 40×Dilution Buffer, 50×ROX Reference Dye, cDNA template, primer, probe and RNase-Free dd$H_2O$ and place it on ice after equilibrated all reagents to room temperature and mixed thoroughly.

Before use, mix each solution evenly by vortex oscillation, and briefly centrifuge to collect the liquid remaining on the tube wall.

# Step 2

It is recommended to prepare Real Time PCR reaction solution on ice according to the following table

| Component | 50 μl system | 25 μl system | 20 μl system | Final concentration |
|---|---|---|---|---|
| 2×SuperReal Color PreMix | 25 μl | 12.5 μl | 10 μl | 1× |
| Forward primer (10 μM) | 1.5 μl | 0.75 μl | 0.6 μl | 0.3 μM |
| Reverse primer (10 μM) | 1.5 μl | 0.75 μl | 0.6 μl | 0.3 μM |
| Fluorescent probe (10 μM) | 1.0 μl | 0.5 μl | 0.4 μl | 0.2 μM |
| cDNA template (including Dilution Buffer) | — | — | — | — |
| 50×ROX Reference Dye | — | — | — | — |
| RNase-Free dd$H_2O$ | To 50 μl | To 25 μl | To 20 μl | — |

# Tips

1. When preparing the quantification mixture, the required reaction volume shall be determined first. Calculate the total volume of reagents required and increase the volume by 10%-20% to compensate for the pipetting loss, thus to ensure the solution is sufficient for desired numbers of reactions. For example, when a total of 5 quantification reactions are required, the number of system preparations is at least 6; when a total of 10 quantification reactions are required, the number of system preparation is at least 11; when a total of 20 quantification reactions are required, the number of system preparations is at least 22. And so on.

2. The required amount of components except cDNA template and $ddH_2O$ should be calculated first according to the number of reactions. . Prepare all components into the same tube on ice, thoroughly mix, and centrifuge for a short time.

3. The required volume of cDNA template and $ddH_2O$ to be added per sample shall be calculated. If the required $ddH_2O$ volume for each sample is the same, the overall required $ddH_2O$ volume can be calculated. Add the $ddH_2O$ to the mixture and mix well completely.

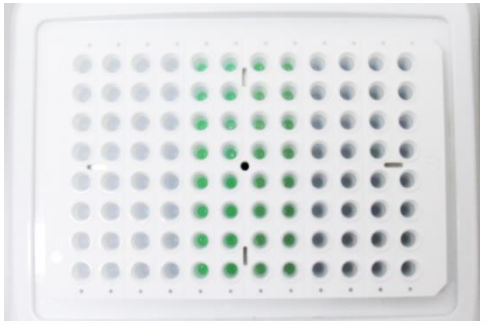
| Reagent | 1 20 μl system Usage amount | 6 20 μl systems Usage amount | 11 20 μl systems Usage amount | 22 20 μl systems Usage amount |
|---|---|---|---|---|
| 2×SuperReal PreMix Plus | 10 μl | 60 μl | 110 μl | 220 μl |
| Forward primer (10 μM) | 0.6 μl | 3.6 μl | 6.6 μl | 13.2 μl |
| Reverse primer (10 μM) | 0.6 μl | 3.6 μl | 6.6 μl | 13.2 μl |
| Fluorescent probe (10 μM) | 0.4 μl | 2.4 μl | 4.4 μl | 8.8 μl |
| 50×ROX Reference Dye | Calculate and add according to the actual situation. | | | |
| RNase-free $ddH_2O$ | Calculate and add according to the actual situation. | | | |

# Tips

4. Dilute and prepare the dilution template according to the following table. The diluent concentration of the final system is 1 ×. The diluent is not an essential component of the reaction. The diluent, premixed reagent and final system are yellow, blue and green respectively.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Additive amount of dilution template in 20 μl PCR system | 1 μl | 2 μl | 2.5 μl | 3 μl | 4 μl | 5 μl | 6 μl |
| Concentration of Dilution Buffer in dilution template | 20× | 10× | 8× | 6.7× | 5× | 4× | 3.3× |
| Required amount of 40×Dilution Buffer in 100 μl dilution template | 50 μl | 25 μl | 20 μl | 16.7 μl | 12.5 μl | 10 μl | 8.4 μl |
| Requied amount of cDNA in 100 μl dilution template | 50 μl | 75 μl | 80 μl | 83.3 μl | 87.5 μl | 90 μl | 91.6 μl |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Additive amount of dilution template in 50 μl PCR system | 2 μl | 2.5 μl | 3 μl | 4 μl | 5 μl | 6 μl | 8 μl |
| Required concentration of Dilution Buffer in dilution template | 25× | 20× | 16.7× | 12.5× | 10× | 8.3× | 6.25× |
| Required amount of 40×Dilution Buffer in 100 μl dilution template | 62.5 μl | 50 μl | 41.7 μl | 31.3 μl | 25 μl | 20.8 μl | 15.6 μl |
| Requied amount of cDNA in 100 μl dilution template | 37.5 μl | 50 μl | 58.3 μl | 68.7 μl | 75 μl | 79.2 μl | 84.4 μl |

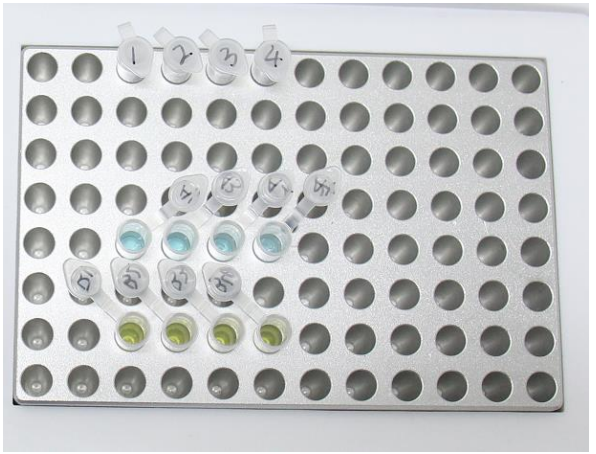


5. Divide the mixture is into each detection tube/well, and add the samples in the order of mixture-template-dd$H_2O$ (if necessary) to prepare the system and thoroughly mix evenly.

# Tips

The method of distributing premixed Mix can effectively improve the repeatability of the experiment. Please operate on ice when preparing and dispensing.

When the final concentration of the primer is 0.3 μM, the amplification conditions are good in most systems. In case of lower amplification efficiency, improve the primer concentration in the PCR reaction system. The primer concentration in PCR reaction system can be appropriately reduced when the non-specific amplification occurs. It can be adjusted in the range of 0.05-0.9 μM if needing to further optimize the primer concentration.

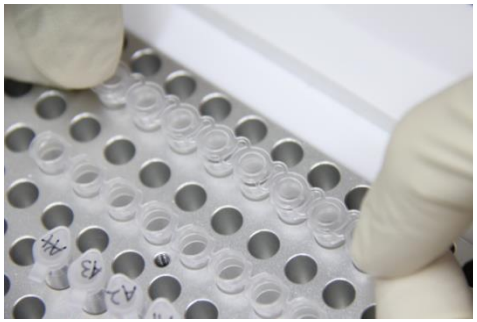


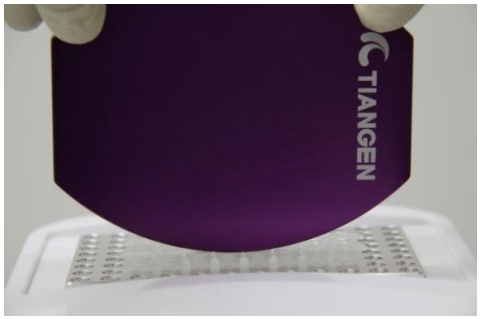
The concentration of the probe is related with the Real-Time PCR thermal cycler, the type of probe and the type of fluorescent labeling substance. Please refer to the instrument instructions or the specific instructions for each fluorescent probe in actual use. When the final concentration of the probe is 0.2 μM, the amplification conditions are good in most systems. It can be adjusted in the range of 0.1-0.5 μM if needing to further optimize the probe concentration.

The optimum ROX Reference Dye concentrations for several common instruments are shown in the following table:

| Instrument | Final concentration |
|---|---|
| ABI PRISM 7000/7300/7700/7900HT/StepOne, etc. | 5×(e.g., 5 μl ROX/50 μl system) |
| ABI 7500, 7500 Fast and ViiA 7;<br>Stratagene Mx3000P, Mx3005P, Mx4000, etc. | 1×(e.g., 1 μl ROX/50 μl system) |
| Roche instrument, Bio-Rad instrument, Eppendorf instrument, etc. | No need to add |

# Step 3







When using the 8-tubes strip, cover the tube cover and compact with a capper after prepared and sub-package of the reaction system. Mark on both ends of the tube cover. Don’t mark the tube cover directly above the detection well, or it will affect the fluorescence reading.

When using the N96 well plate, seal the plate with plate cover and compacted after prepared and sub-package of reaction system, and the marks shall be marked around the well plate or the detection well without samples.

# Step 4




Use a plate centrifuge to briefly centrifuge N96 well plate or 8-tube strip. Please note to make the bottom of the plate outward and keep balance.

Place the 8-tube strip on the tube rack and fix it with a fixing piece to centrifuge when centrifuging the 8-tube strip.

# Step 5

| Stage | Circles | Temperature | Time | Content | Fluorescence signal acquisition |
|---|---|---|---|---|---|
| Initial denaturation | 1× | 95℃ | 15 min | Initial denaturation | No |
| PCR reaction | 40× | 95℃ | 3 sec | Denaturation | No |
| | | 60-66℃ | 20-32 sec | Annealing/Extension | Yes |

Transfer the samples to the real time PCR instrument and set the program to start the qPCR reaction.