Operation Guide of SuperReal PreMix Plus (SYBR Green) (FP205)

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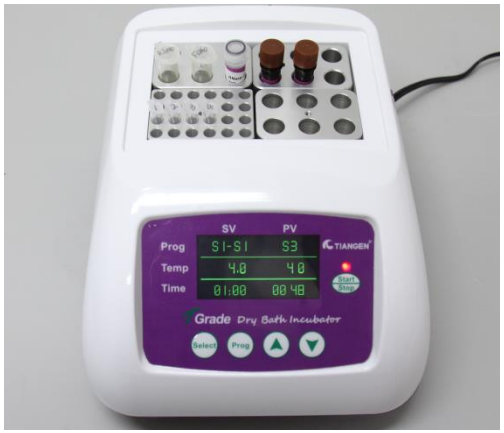
Experiment Preparation

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 PreMix Plus, 50×ROX Reference Dye, cDNA template, primer and RNase-Free ddH_2O and place it on ice after equilibrating all reagents to room temperature and mixing 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 PreMix Plus	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
cDNA template	—	—	—	—
50×ROX Reference Dye	—	—	—	—
RNase-free ddH_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.

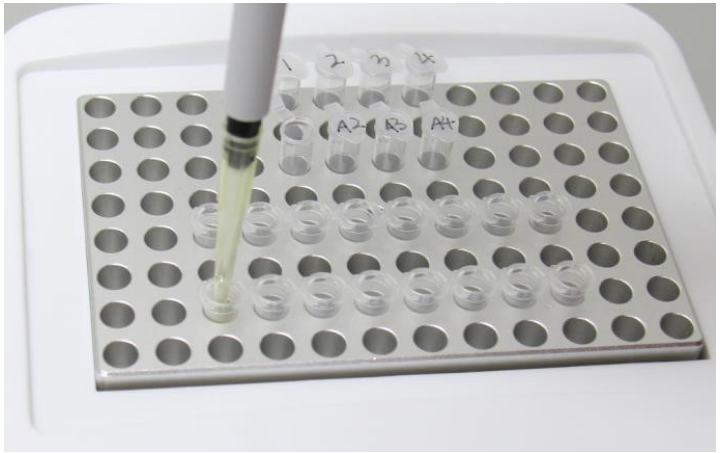
3. The required volume of cDNA template and ddH_2O to be added per sample shall be calculated. If the required ddH_2O volume for each sample is the same, the overall required ddH_2O volume can be calculated. Add the ddH_2O to the mixture and mix well completely.

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
50×ROX Reference Dye	Calculate and add according to the actual situation.			
RNase-free ddH_2O	Calculate and add according to the actual situation.			

4. Divide the mixture into each detection tube/well, and add the samples in the order of mixture-cDNA-ddH_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.

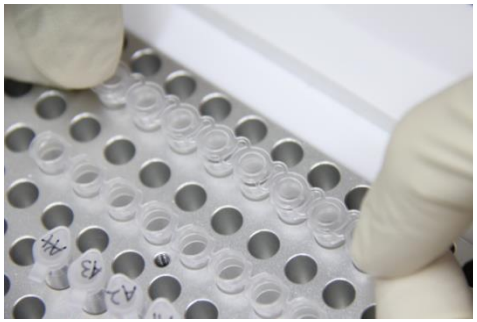


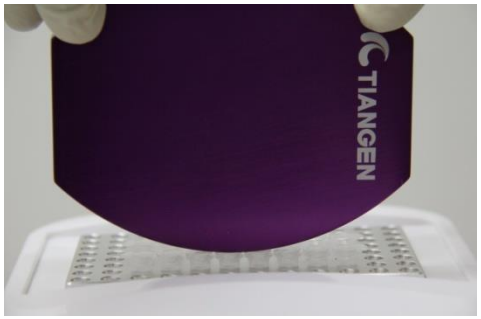
When the final concentration of the primer is 0.3 μM, the amplification conditions are good in most systems. In case of lower amplification efficiency, improve the primer concentration in the PCR reaction system. The primer concentration in PCR reaction system can be appropriately reduced when the non-specific amplification occurs. It can be adjusted in the range of 0.2-0.5 μM if needing to further optimize the primer 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; 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℃	10 sec	Denaturation	No
		60-66℃	20-32 sec	Annealing/Extension	Yes
Melting Curve Analysis (Melting/Dissociation Curve Stage)					

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