SYBR Green qPCR Fast 2X Master Mix

FISHER MOLECULAR BIOLOGY

Description

Real-time Quantitative PCR (qPCR) is a powerful technique in detecting initial DNA input in a PCR reaction by fluorescence signal accumulation. The DNA double strand bonded dye, SYBR® Green I is the most commonly used dye in qPCR. 2X SYBR Green Fast qPCR Mix is provided in 3 versions: No Rox, Low ROX, High Rox and they are optimized for Real Time machines with no Rox, High Rox and L ow ROX mode. It contains all required components in qPCR except primers and template. It is convenient for experiment and suitable for multiple species. The above features make it as an ideal experiment tool for gene quantitative research.

Compatible Instruments (table 1.)

Following table is helpful for choosing the right product formats

No Rox Reference Dye I	Bio-Rad iCycler serious, Roche Light Cycler serious Qiagen/Corbett serious and others
Low Rox	ABI 7500, ABI ViiATM7, ABI QuantaStudio serious, Stratagene serious, Corbett Rotor Gene 3000 and others
High Rox Reference Dye	ABI 7000/7300/7700/7900, ABI StepOne/StepOnePlus, Eppendorf and others

Kit Contents

Cat.n.	Description	Size
FS-T-50212-5	2X SYBR Green Fast qPCR Mix *(No ROX)	5 X 1 mL
FS-T-50213-5	2X SYBR Green Fast qPCR Mix *(Low ROX)	5 X 1 mL
FS-T-50214-5	2X SYBR Green Fast qPCR Mix *(High ROX)	5 X 1 mL

1 ml =100 reactions

Including Hot Start Taq DNA Polymerase, Mg^2, dNTPs,Sybr Green I

Advantages:

- SYBR Green I fluorescence combined with double-stranded DNA has high sensitivity and SNR.
- Compatible with fluorescence quantitative PCR instruments requiring high concentration of ROX;
- Hot-start Taq DNA polymerase was used to improve specificity.
- Premixed reagent is easy to operate;

Materials Required

1. EP tubes, PCR tubes and other related materials.
2. qPCR specific primers and templates.
3. qPCR plates and seal membrane.

Usage Notes

1. Before using 2X SYBR Green Fast qPCR Mix, please make sure that the mix is thawed completely and then placed it on ice for use.
2. Mix other qPCR components with Mix thoroughly and gently by pipetting or vortexing. After usage, place the Mix back to -20°C for long time storage or 4°C for short period storage.
3. 2X SYBR Green Fast qPCR Mix contains Hot Start *Taq* polymerase, all operation should be performed on ice.
4. 2X SYBR Green Fast qPCR Mix contains ROX dye, suits for all qPCR instruments (see table 1)
5. To avoid contamination, pipette tips with filters is suggested.
6. To guarantee better qPCR results, DNA template in good quality is suggested.

Before Use

1. Specificity of primers should be checked and a final concentration of 0.2 μM is suitable for most of primers.
2. The length of amplification products is usually range from 70 bp to 200 bp.
3. Dilute the template in gradient.
4. Add 1 pg-50 ng DNA as PCR templates and a "No Template Control sample" is suggested.
5. To ensure the confidence of experiment, at least 2 repeats of each sample is suggested.

Procedure

1. Prepare the following reaction systems on ice for a 20 ul

Component	20 ul Reaction
2X SYBR Green Fast qPCR Mix	10 μL
Forward Primer (10 μM)	0,4 μL
Reverse Primer (10 μM)	0,4 μL
gDNA or cDNA (<50 ng)	2 μL
RNase free ddH_20	up to 20 μL

2. Dissolve 2X SYBR Green Fast qPCR Mix (No Rox,Low ROX, High ROX) at room temperature then placed it on ice for further usage. Before using, agitate the Mix thoroughly and centrifuge to collect all solution.
3. Calculate the amount of mix need, generally a 10% extra amount is suggested.
4. Dispense solution in sterile PCR or EP tubes in case of any contamination.
5. Add all components listed in above table, agitate the tubes gently to mix thoroughly (avoid bubbles) and centrifuge it.
6. Dispense the reaction solution into qPCR plates and seal the plates with optical membrane.
7. 2500 rpm centrifuge the qPCR plates to collect all solution.

2. Program qPCR reaction as follows:

Stage 1	Denaturation	Reps:1	95°C	3 minutes
Stage 2	Cycles	Reps: 40-45	95°C	5 seconds
			60°C	30-34 seconds
Stage 3	Melt Curve	Reps: 1	Default	

**Confirm there is a signal collection step after each extending step. The extending time is varied according to different machines: 30s for StepOne Plus, 31s for 7300 and 34s for 7500*

Data Analysis :

1. Draw a standard curve according to Ct values of endogenous gene. The value of R^2 should be more than 0.98 and the slope of curve should be in the range of -3 to -3.5 which means the PCR amplification efficiency is in the range of 90% to 120%.
2. The standard deviation (STD) of Ct values should be less than 0.2 and the variation of Ct value for different experiment should be less than 0.5 (the threshold value of different experiments should be same when comparing Ct values).
3. The single melt curve indicate the no non-specific amplification products or primer dimmers, and theTm value in melt curve is usually in the range of 80 to 95°c.

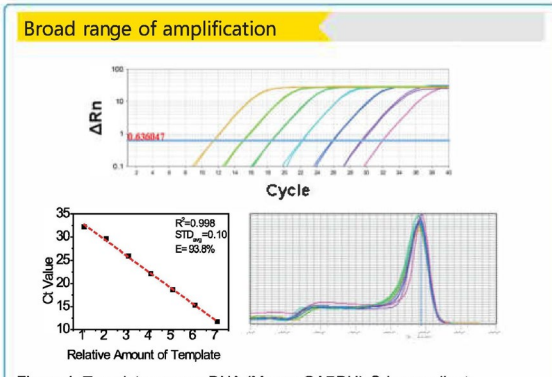

Figure 1. Template: mouse DNA (Mouse GAPDH), 6-log gradient dilution. The target gene GAPDH was detected by Fisher SYBR Green qPCR Fast 2X Master Mix. The experimental results show that the qPCR reagent can be accurately amplified between12-32 Ct, showing good amplification ability.

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Melt Curve Show Multiple Peaks	**a**. Primer Design : Design the primer following basic primer design protocols. b. Primer Concentration Too High: lower down the concentration of primers
Unusual Amplification Curves	**a.**Amplification Curve Not Smooth: Too low amplification signal, increase the template input and make sure the qPCR Mix is stored properly. **b**. Inconsistent Amplification Curve Bubbles causes abnormal qPCR results, centrifuge the plate prior to running it. **c.** Abnormal Amplification Curves: the default baseline value of machine is set to be from 3 to 15, the baseline setting can be changed according actual amplification conditions. Besides, the degradation of template may affect the curve.
No Amplification Curves after Reaction	**a.**Not Enough PCR Cycles : the PCR cycle number is usually set to be 40. It should be noted a higher cycle number may increase the background signal. **b.**Primer Degradation : Use electrophoresis to confirm the Integrity of primers. **c.**Confirm the Signal Collection Step : the signal collection step are usually set to be after the annealing-extending step for two-step qPCR and after extending step for Three-step qCPR. **d.**Template Input Too Low: Increase template concentration or add extra repetition.
Template Degradation	Use freshly prepared template (Use electrophoresis to confirm Integrity of template)
Not Enough Initial Denaturation Time:	2X SYBR Green Fast qPCR Mix uses Hot-Start Taq polymerase, the pre-denaturation time should be at least 3min.
Ct Value Too Late	**a**.Low Amplification Efficiency : Optimize reaction condition or change primer. **b**.Template Input Too Low : Increase template concentration or add extra repeat **c**.Template Degradation : Use freshly prepared template(Use electrophoresis to confirm Integrity of template) **d.**Too Long PCR Products : The length of amplification products is usually in the range of 70 bp-200 bp. **e.**PCR Inhabitation Reagent : use new template or dilute the template. **f.**Too Short Pre-denaturation Time : 2X SYBR Green Fast qPCR Mix contains Hot-Start Taq polymerase, the pre- denaturation time should be at least 3min.

5) NTC Shows Amplification	**a.** Contamination: Use sterile water to conduct experiment and the all operation is suggested to be done in clean room to avoid aerosol contamination. **b.**Non-Specific PCR Products: analyze with melt curve.
6) Inconsistent Results	**c.Inconsistent Sample Added:** Use proper pipetting techniques **d.Inconsistent Temperature in qPCR Machine:** ensure periodic machine calibration. **e.Template Concentration Too** Low: the lower template input, the poorer qPCR result is. Increase the template concentration**.** **f. Inconsistent Threshold Set:** when comparing the qPCR results in different plates, make sure the threshold value of each experiment is the same.