FISHER MOLECULAR BIOLOGY

FS-T-50215 2X Universal SYBR Green Fast qPCR Mix

Size: 5 ml / 25 ml

Description	FS-T-50215-5	FS-T-50215-25
2X Universal SYBR Green Fast qPCR Mix	500 RXN 5 X 1 mL	2,500 RXN 25 X 1mL

1 ml= 100 reactions

Introduction

Real-time Quantitative PCR (qPCR) is a powerful technique in detecting initial DNA input in a PCR reactionby fluorescence signal accumulation. The DNA double strand bonded dye, SYBR® Green I is the most commonly used dye in qPCR. It contains Hot Start Taq, to avoid unexpected amplification Results. It is an optimized qPCR Mix you need to add 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.

Instruments

2X Universal SYBR Green Fast qPCR Mix contains the novel designed universal reference dye, which can realize higher signal resolution and suits for all qPCR Instruments (including High ROX mode, Low ROX mode and No Rox mode).

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 Universal 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.
3. 2X Universal SYBR Green Fast qPCR Mix (No ROX) contains Hot Start *Taq* polymerase, all operation should be performed on ice.
4. 2X Universal SYBR Green Fast qPCR Mix contains specially reference dye, which suits for all qPCR instruments . No Rox is required.
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 tor 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 samples is suggested.

PROCEDURE:

1. Prepare the following reaction systems on ice

Components	20 ul Reaction
2X Universal SYBR Green Fast qPCR Mix (No ROX)	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 ddH20	to 20 μL

(1) Dissolve 2X Universal SYBR Green Fast qPCR Mix (No ROX) at room temperature then placed it on ice for further usage. Before using, agitate the Mix thoroughly and centrifuge to collect all solution.
(2) Calculate the amount of mix need, generally a 10%extra amount is suggested.
(3) Dispense solution in sterile PCR or EP tubes in case of any contamination.
(4) Add all components listed in above table, agitate the tubes gently to mix thoroughly (avoid bubbles) and centrifuge it.
(5) Dispense the reaction solution into qPCR plates and seal the plates with optical membrane.
(6) 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 morethan 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 the Tm value in melt curve is usually in the range of 80 to 95°c.

TROUBLESHOOTING

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 Universal SYBR Green Fast qPCR Mix (No ROX) uses Hot-Start Taq polymerase, the pre-denaturation time should be at least 3min.
Ct Value Too Late	a. **Low Amplification Efficiency**：Optimizereaction condition or change primer. b. **Template Input Too Low**：Increase templateconcentration or add extra repeat c. **Template Degradation**：Use freshly prepared template (Use electrophoresis to confirmIntegrity of template) d. **Too Long PCR Products**：The length of amplification products is usually in the range of70 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 (No ROX) 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. 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.

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