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FS-T-1200-HR SYBR Green qPCR Master Mix (High Rox)

Product Description

SYBR Green qPCR Master Mix is a ready-to-use cocktail containing all components except primers and template.

The 2X master mix contains Taq DNA polymerase, dNTPs, MgCl2, SYBR Green I, Rox or No Rox and stabilizers.

In the formulation, for **Hot Start,** Taq DNA Polymerase is chemically modified and its activity is completely blocked until the first denaturation step in PCR program. This eliminates spurious amplification products resulting from non-specific priming events during reaction setup and initiation, and increases overall reaction efficiency.

For easy and avoiding potential error manipulation, the products are provided in three formats:

- no Rox,
- low Rox,
- high Rox.

Customers could choose suitable product format based on their qPCR instruments used.

FS-T-1200-NR contains no ROX,
FS-T-1200-LR contains low ROX，
FS-T-1200-HR contains high ROX.

Following table is helpful for choosing right product formats

No ROX	Bio-Rad CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4; Cepheid SmartCycler®; Eppendorf Mastercycler® EP Real plex, Realplex 2 s; Illumina Eco qPCR; Qiagen/Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene 6000; Roche Applied Science LightCycler™ 480; Thermo Scientific Piko Real Cycler
Low ROX	Applied Biosystems 7500, 7500 Fast, ViiA7; Stratagene MX4000, MX3005P, MX3000P
High ROX	Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne, StepOnePlus.

Ordering information:

Cat. N°	Size	Cat. N°	Size
FS-T-1200-NR	1 ml	FS-T-1200-NR-5	4X1.25 ml
FS-T-1200-LR	1 ml	FS-T-1200-LR-5	4X1.25 ml
FS-T-1200-HR	1 ml	FS-T-1200-HR-5	4X1.25 ml

Experimental Procedure

1. Set up reaction in qPCR tube as follow:

Composition:	20 µl reaction system
2 X SYBR Green qPCR Master Mix	10.0 µl
Primer1 (10 µM)	0.4 µl
Primer2 (10 µM)	0.4 µl
Template DNA/cDNA	X µl
ddH2O	To

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Storage Condition:

Store at -20°C avoiding light, the master mix should be not stored at 4°C for longer time after being thawed. The mix can tolerate 30 cycles freeze and thaw without affecting Ct value for sample analysis, keep at -20°C, if no plan to use in certain time.
Please blending the master mix before aliquot.

Quantity of components in the reaction could be adjusted as needed to achieve better results:

1) Generally 0.2 μM primer concentration is suitable, but when results are not satisfied, trying primer concentration between 0.1-1.0 μM range
2) qPCR method is very sensitive, accuracy of added temple is essential, recommending using diluted templates to reduce to increase aliquot accuracy, and the result is reproducible.
3) If templates are undiluted cDNA from standard reverse transcription reaction, the volume of template is no more than 10% of the reaction volume.

2. Running qPCR reaction as following

Stage 1	Pre-denatue	Reps: 1	95°C	5-10 min
Stage 2	Cycling	Reps: 40	95°C 60°C	10 sec 30 sec
Stage 3	Melting curve	Reps: 1	95°C 60°C 95°C	15 sec 60 sec 15 sec

2.1 Pre-denature condition is suitable for most of reactions, if templates are complicated, extend to 10 min.
2.2 for less 300 bp fragment amplification, 30 second extending time is enough, for large than 300 bp fragment amplification, 60 second extending time is recommended.
2.3 Melting curve collecting program depends on instrument's model, please choose acquiescence for the model

Quality Control

Purity detection：all components are analyzed without exo- and endo-nuclease and nuclear acid.

Optimizing reaction

Best reaction condition should have following characteristic: single melting curve, amplification efficiency is almost 100%, lower Ct value (high amplification efficiency), if reaction is not as expected under acquiescence condition, reaction condition could be optimized as following ways.

1. Primer concentration and reaction: when primer concentration is between 0.1～1.0 μM, higher primer concentration leads non-specific amplification, but amplification efficiency is increased.

2. Amplification program and reaction: To increase amplification specificity, increase annealing temperature and extending amplification time

Two step standard program	Increase annealing temperature (3℃ each time)	Two step standard program	Increase extending temperature
95℃/10 sec 60℃/30 sec	95℃/10 sec 63℃/30 sec	95℃/10 sec 60℃/30 sec	95℃/10 sec 60℃/60 sec

3. To increase amplification efficacy, change two step amplification to three step and increase extending time.

Three Step Program	Increase extending time
95℃/10 sec 56℃/30 sec 72℃/30 sec	95℃/10 sec 56℃/30 sec 72℃/60 sec

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Frequently Met Problems and Solving Ways

1，Amplification Plot is Strange

- ✓ Amplification plot is not smooth：signal is weak，generated by program correct. Increase template concentration and repeat experiment；ROX format error, please check your instrument model.
- ✓ Amplification curve is broken and down: template concentration is high, and baseline value is higher than CT value. Reduce baseline endpoin t(CT value-4) and re-analyzing the data
- ✓ some amplification curve is down: There is a bubble in the reaction,please check before running

2. NO Amplification Plot after Running

- ✓ Running cycle is not enough：Usually 40 cycles are used, but more cycle could increase background signal and reduce data reliability.
- ✓ Confirm signal collecting step: For two step program, data collecting is set up on annealing and extending step,for three step program, data collecting is set up at 72℃ extending step
- ✓ Confirm primer upgraded: Primers not used for a long time, check by running PAGE gel to confirm primer integrity.
- ✓ Template concentration is too low: Reduce dilution folds and repeat experiment, for unknown sample,trying highest concentration first
- ✓ Degradation of template：Re-prepare template and repeat the experiment

3. CT value appears too low:

- ✓ Amplifcation efficiency is too low: Optimizing reaction condition and trying three step program or redesigning primers
- ✓ Template concentration is too low: Reduce sample dilution folds and repeat the experiment, try highest template concentration first
- ✓ Size of PCR product is too long: Recommending size for amplification is between 80 bp-150 bp
- ✓ PCR inhibitors existing in the reaction: Usually inhibitors are introduced by template, using more dilutionfolds or re-prepare template and repeat experiment

4，Negative control shows obvious amplification:

- ✓ Reaction reagents are contaminated：Change new mix, water, primers and repeat experiment, set reaction in hood to reduce aerosol contamination.
- ✓ Primer dimer appear： Analysis with melting curve

5，For absolute quantification, linear characteristic of standard curve is not satisfied

- ✓ Aliquot error: Increase template dilution folds and increase reaction volume
- ✓ Standard curve samples degraded： Re-prepare standard curve sample.
- ✓ Template concentration is too high： Dilute samples more folds.

6，Multiple peaks for melting curve

- ✓ Primer design is not optimized: Re-design primers.
- ✓ Primer concentration is too high: Reduce primer concentration reasonably
- ✓ cDNA sample contaminated with genomic DNA: Re-prepare RNA and remove genomic DNA before reverse transcription to make cDNA.

7， Poor experiment repeatability

- ✓ Aliquot is accurate: Use brand Pipette: Dilute template more fold and increase adding volume
- ✓ Control of temperatures are different position for QPCR instrument: Calibrate the instrument in certain time
- ✓ Template concentration is too low: The lower template concentration, the poorer repeatability. Reduce dilution of template and increase adding volume.

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