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SYBR GREEN qPCR 2x MASTER Mix FS-T-1200

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, $MgCl_2$, 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:

CAT.#	Description	SIZE
FS-T-1200-NR	Sybr Green qPCR 2x Master Mix No Rox	1 ml
FS-T-1200-LR	Sybr Green qPCR 2x Master Mix Low Rox	1 ml
FS-T-1200-HR	Sybr Green qPCR 2x Master Mix High Rox	1 ml

1 ml: 100 reactions

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.

Procedure

3. Set up reaction in qPCR tube as follow:

Composition	20 µl reaction system
SYBR Green qPCR 2x Master Mix	**10 µl**
Primer 1 (10 µM)	**0.4 µl**
Primer 2 (10 µM)	**0.4 µl**
Template DNA/cDNA	**X µl**
ddH2O	**up to 20 µl**

Suggestions for 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 toincrease 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.

Storage: at -20°C avoid light, After thawing cycle the Master Mix should be stored at 4 °C for long time.
The Mix should be kept a -20°C , before using just blend the Master Mix.

Running qPCR Reaction as follows:

Stage	Step	Reps	Temperature	Time
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, 60second extending time is recommended.
2.3 Melting curve collecting program depends on instrument's model, please choose acquiescence for the model.

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 program

Two step standard program 95°C/10 sec 60°C/30 sec	Increase annealing Temperature(3°C each time) 95°C/10 sec 63°C/30 sec
Two step standard program95℃/10 sec 60℃/30 sec	Increase extending temperature 95°C/10 sec 60°C/60 sec

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

THREE STEP program

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

Quality Control: Purity detection: all components are analyzed without exo - endo-nuclease and nucleic acid

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