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FS-T-50111 SUPER GREEN qPCR 2X Master Mix

Kit Contents

Description	CAT.#	Sizes
Super Green qPCR 2x Master MIX	**FS-T-50111**	1 mL*

*1 mL = 100 reactions

The kit contains two components:

Component A : (1 single Vial) contains Super Green dye, dNTP, PCR buffer (including Tris and MgCl2) and Hot-start Taq polymerase.

Description:

Super Green qPCR 2x Master Mix is a ready-to-use hot-start mix for qPCR and DNA melt curve analysis of PCR amplicons. It is formulated for qPCR using a fast-cycling protocol, but also can be used for qPCR with regular cycling protocols.

Super Green dye is a unique DNA-binding dye with features ideal for both qPCR and melt curve analysis. Green dye binds to dsDNA via a novel "release-on-demand" mechanism, which permits the use of a relatively high dye concentration in qPCR without PCR inhibition.

Super qPCR 2x Master Mix contains our proprietary chemically-modified Hot-Start Taq DNA Polymerase. Unlike AmpliTaq Gold, which is also a chemically modified Taq but takes 10 minutes or longer to activate, this Taq DNA Polymerase is fully activated in 2 minutes with high activity recovery, making it particularly suitable for fast PCR. HS-Taq is completely inactive at room temperature and largely free of DNA contamination. This makes HS-Taq superior to any antibody-based hot-start Taq, which is typically not completely inactive at room temperature and is prone to DNA contamination due to the nature of antibody production.

A unique feature of **Super Green dye** is its safety. DNA-binding dyes are inherently dangerous due to their potential to cause mutation. With this in mind, our scientists designed ***Super Green dye*** such that it cannot cross cell membranes, thus preventing the dye from being in contact with genomic DNA in live cells.

An added benefit of **Super Green qPCR 2x Master Mix** is that you can analyze your PCR product by gel electrophoresis without the need to add another DNA-binding dye to either your loading buffer or gel. The Super Green dye in the Master Mix can act as a DNA pre stain, permitting direct visualization of DNA bands following electrophoresis.

Spectral Properties: λabs/λem = 500/530 nm (bound DNA) λabs = 471 nm (without DNA)

Reaction Conditions PCR

Reagents	20ul reaction	Final conc.
Super Green qPCR 2x Master Mix	10.ul	1X
10 um Forward Primer	X ul each	0.1~0.5 uM
10 um Reverse Primer	X ul each	0.1~0.5 uM
Template**	Variable (see note 1 and 2)	NA
Water RNase Free	Up to 20ul	

Notes

1. **cDNA templates: Super Green qPCR 2X Master Mix** is suitable for mRNA quantitation if a two-step procedure is followed. The first step involves converting the mRNA to cDNA by reverse transcription (components not provided). A portion of the synthesized cDNA can then be quantitated by using Super Green qPCR 2X Master Mix in the second step. To ensure optimal amplification efficiency, the aliquot of the cDNA sample to be amplified should not exceed 10% of the volume of the PCR reaction. We recommend cDNA synthesis kits . For accurate quantitation of transcript levels, a no-RT control is recommended to check for possible genomic DNA contamination.
2. **One-step RT-qPCR** can also be applied for mRNA quantitation. Primer sets must be well characterized to ensure no primer-dimer formation. We recommend that you titrate the amount of reverse transcriptase and the duration of the RT step. If possible, design primers to have Tm at 55 ℃, run both RT step and extension step at 55 ℃. For accurate quantitation of transcript levels, a no-RT control is recommended to check for possible genomic DNA contamination.
3. **Template concentration**: The optimal amount of template DNA varies by application. Recommended amounts of genomic DNA template per reaction typically range from 50 pg to 50 ng per reaction. Recommended amounts of cDNA typically range from 50 fg to 50 pg, based on the amount of input RNA in the RT reaction.
4. **ROX reference dye**: For certain instruments, ROX is necessary for accurate Ct determination from well to well. Refer to Table 1 (See Page 5-6) for the recommended ROX concentration for your instrument. ROX may add noise to melt curve analysis, which could be mistaken for real peaks. Thus, in case of unexpected peaks, uncheck "ROX" in the "Passive Reference Dye" box in the software so that data is not collected from the ROX fluorescence channel, then re-analyze the data.

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Cycling Protocols

You may choose one of the following three protocols, depending on the nature of your amplicon and instrument capability.

A. Universal cycling protocol

This cycling protocol can be used on nearly all qPCR instruments. The protocol also may be useful for targets that are relatively difficult to amplify underfast cycling conditions.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme Activation	**95 ℃**	**2 min**	**1**
Denaturation Annealing & Extension	**95 ℃** **60 ℃**	**15s** **60s**	**45**

B. Two-step fast cycling protocol

This cycling protocol should be applicable to most amplifications where the primer Tm's are designed to be 60℃. Melt curves may be performed by following instructions provided for your instrument.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme Activation	95 ℃	2 min	1
Denaturation **Annealing & Extension**	95 ℃ 60 ℃	5s (Note 5) 30s	45

Note

5. Denaturation time: The holding time for denaturation can be lower than 5 seconds, including as low as 0 second, if you have a relatively short amplicon. When the denaturation time is set to "0" in the program, it merely means that the temperature is ramped up to 96℃ and then immediately ramped down with no stay. Setting the time to 5 s will ensures a more robust denaturation for relatively long or high GC amplicons. Instruments with fast ramping capability further add reliability to amplicon denaturation.

C . Three-step fast cycling protocol

This cycling protocol can be used if you would like to have the extension step to be performed at a higher temperature than the annealing step. For example, if you have relatively long primers that tend to anneal non-specifically, carrying out the extension step at a higher temperature can reduce nonspecific amplification. Melt curves may be performed by following instructions provided for your instrument.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme Activation	95 ℃	2 min	1
Denaturation **Annealing** **Extension**	95 ℃ 50~60 ℃ 72 ℃	10 s 10s (Note 6) 10s (Note 7)	45

Notes

6. Annealing temperature: The annealing temperature should be set at your primer Tm, which should generally be 50-60℃ for optimal result. However, whenever possible, primer Tm (and thus extension temperature) should be designed closer to 60℃ (but still within 50-60℃ range) to minimize the gap between annealing and denaturation temperatures. This way, the temperature ramping will take less time, which in turn facilitates amplification.

7. Extension temperature: Extension at 72℃ is usually more efficient for most amplicons. However, for AT-rich amplicons (>70% AT) or amplicons that have an AT-rich patch, extension at 60℃usually gives better results.

PCR Machines requiring ROX dye

Table 1. Recommended ROX Concentration for PCR Instruments

PCR Instrument	Recommended ROX concentration	Amount of 10× ROX per 20 µL Reaction
BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96,CFX-384, MJ Opticon, Option2, Chromo4, MiniOpticon Qiagen: Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000 Eppendorf: Mastercycler realplex Illumina: Eco RealTime PCR SystemCepheid: SmartCyler Roche: LIghtCycler 480, 96, LightCycler 2.0	**No ROX**	None
ABI: 7500, 7500 Fast, ViiA 7 Stratagene: MX4000P, MX3000P MX3005P, QuantStudio, Illumina Eco, Thmorgan Q6,Q4	**Low ROX 0.05~0.1× (final)**	**Dilute 10× ROX 1:10 withdH_2O to obtain 1× ROX; add 1 to 2 uL of 1× ROX per 20 uL react.**
ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus	**High ROX 1×(final)**	**2 uL of 10× ROX per 20 uL reaction**

General Considerations

1. qPCR instruments: For iCycler users, you do not need to add FAM to your PCR mix as Super Green dye has a slight background fluorescence that provides adequate and stable baseline level fluorescence. For Roche LightCycler users using glass capillaries for reactions, you need to add BSA to your PCR reactions (~0.5 mg/mL final concentration). BSA is not necessary if transparent plastic capillary tubes are used.

2. Instruments for melt curve analysis: Suitable instruments include Rotor-Gene 6000, ABI 7500 FAST and HR1™, 384-well LightScanner™ and Roche LightCycler 480. Rotor-Gene 6000, ABI 7500 FAST and Roche LightCycler 480 are capable of performing both qPCR and melt curve analysis. Follow the manufacturer's instruction for data collection and analysis.

3. Expected ΔR and ΔRN: When comparing signal strength among various commercial qPCR Master Mixes, one needs to be mindful of the method used in the comparison. Conventionally, ΔR is the fluorescence gain above the baseline. In general, 10 µL of 1× Super Green reaction generates higher ΔR than 50 µL 1× PowerSYBR from ABI or 1× SYBR Green ER from Invitrogen. ΔRN is defined as ΔR divided by the signal in the ROX channel. Therefore, a higher concentration of ROX will generate smaller ΔRN. ΔRN will also become smaller when ROX is excited at its maximal as in the case of ABI 7500, iCycler IQ, MJ opticon, MJ Chromo4, MX3000, and MX4000. Accordingly, the lower ROX concentration used in some commercial SYBR Green Master Mixes will produce a higher ΔRN.

4. Expected kinetic curve: Based on our comparative studies, amplification curves of Super Green qPCR 2x Master Mix generally are more robust than other commercial Master Mixes formulated using SYBR Green I. Because of SYBR's inhibitory effect, SYBR-based Master Mixes may tend to stall amplification 5-7 cycles after the signal reaches the Ct threshold. In contrast, reactions in Super Green qPCR 2x Master Mix can continue to amplify for as many as 50 cycles.

5. Expected Ct value: Under similar conditions, Ct values generated by Super Green and SYBR Green I may differ from each other by +1 or –1.

6. Amplicon length: To maximize amplification efficiency with Super Green qPCR 2x Master Mix, the optimal amplicon length is 50-200 bp. For longer amplicons you may need to extend the elongation time.

7. Gel electrophoresis analysis of PCR product: To analyze your PCR product by gel electrophoresis using Super Green dye in the Master Mix as a prestain, simply add DNA loading buffer your PCR reaction solution, load on a gel, and conduct electrophoresis as usual. No additional DNA-binding dye needs to be added to either the loading buffer or the gel. Gel visualization can be carried out using a 254 nm UV box, or a gel imager or Dark Reader using a SYBR Green filter. Alternatively, the gel may be imaged using a 488 nm laser-based gel scanner.

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