

Fisherbrand™ SYBR™ Green qPCR Master Mix

Catalog Numbers A59528, A59529, A59530

Pub. No. MAN0028448 Rev. A.0

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *Fisherbrand™ SYBR™ Green qPCR Master Mix User Guide* (Pub. No. MAN0028447). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

See the *Fisherbrand™ SYBR™ Green qPCR Master Mix User Guide* (Pub. No. MAN0028447) for the following information:

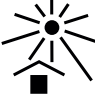
- Limited warranties for consumables and general labware
- Exclusions
- Limitations
- Remedies

Contents and storage

Fisherbrand™ SYBR™ Green qPCR Master Mix

Cat. No.	Amount	Number of 20-μL reactions	Storage
A59528	1 mL	100	–30°C to –10°C
A59529	5 mL	500	
A59530	50 mL	5,000	

Symbols

Symbol	Description	Symbol	Description
	CATALOGUE NUMBER		BATCH CODE
	USE-BY DATE		KEEP AWAY FROM SUNLIGHT, KEEP AWAY FROM HEAT
	TEMPERATURE LIMIT		MANUFACTURER
	READ SAFETY DATA SHEET (SDS)		

Guidelines

For detailed instructions and ordering information for additional products, see the *Fisherbrand™ SYBR™ Green qPCR Master Mix User Guide* (Pub. No. MAN0028447).

Requirements for input DNA

Use 1–10 ng of cDNA or 10–100 ng of gDNA per reaction. See the recommendations in your reverse transcription kit for more information.

Guidelines for PCR reactions

- Four replicates of each reaction are recommended.
- Reaction mixes can be prepared depending upon experimental requirements. Scale the components according to the number of reactions and include 10% overage.
- If using smaller reaction volumes, scale all components proportionally. Reaction volumes of less than 10 µL are not recommended.
- The recommended final primer concentration for primers with a T_m of 55°C is 400 nM.

Guidelines for no-template control reactions

No-template control (NTC) reactions can be used to identify PCR contamination. NTC reactions contain all of the reaction components except for the sample.

Prepare the PCR reactions

1. Combine the master mix, the primers, DNA template, and nuclease-free water, according to the following table.

Component	Final concentration	Volume for 1 reaction (10-µL reaction)	Volume for 1 reaction (20-µL reaction)
Fisherbrand™ SYBR™ Green qPCR Master Mix	1X	5 µL	10 µL
Forward and reverse primers ^[1]	400 nM	0.5 µL	1 µL
DNA ^[2]	0.5 ng/µL	1 µL ^[3]	2 µL ^[4]
Nuclease-free water	—	3.5 µL	7 µL
Total PCR volume	—	10 µL	20 µL

^[1] The final primer concentration can vary from 300–800 nM. A final concentration of 400 nM is recommended for primers with a T_m of 55°C.

^[2] Use 1–10 ng of cDNA or 10–100 ng of DNA. See the recommendations in your reverse transcription kit for more information.

^[3] Does not exceed 4.5 µL.

^[4] Does not exceed 9.0 µL.

2. Mix the components thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.
3. Transfer the appropriate volume of each reaction to each well of an optical PCR plate.
4. Seal the plate with an optical adhesive cover, then centrifuge briefly to collect the contents at the bottom of each well and eliminate any air bubbles.

PCR can be performed on the reaction plate up to 72 hours after completing the set-up, when stored at room temperature protected from light.

Note: Pre-PCR stability is influenced not only by the master mix, but also by the target being analyzed. For maximum confidence, confirm stability profiles of the specific targets.

Set up and run the real-time PCR instrument

1. Set up the thermal protocol, according to the following table.

Note: Standard cycling conditions are recommended for genomic DNA templates or long amplicons.

Step	Temperature	Duration		Cycles
		Fast cycling mode	Standard cycling mode	
UDG activation	50°C	2 minutes	2 minutes	1
Enzyme activation	95°C	2 minutes	2 minutes	1
Denature	95°C	5 seconds	15 seconds	40
Anneal/extend	60°C	30 seconds	60 seconds	

2. Set the instrument to perform a default dissociation step, according to one of the following tables.

Note: Select the same mode used in step 1.

Fast cycling mode

Step	Ramp rate ^[1]	Temperature	Time
1	1.99°C/second	95°C	15 seconds
2	1.77°C/second	60°C	1 minute
3 (Dissociation)	0.075°C/second	95°C	15 seconds

^[1] For the 7500 Real-Time PCR System, use the default ramp rate.

Standard cycling mode

Step	Ramp rate ^[1]	Temperature	Time
1	1.6°C/second	95°C	15 seconds
2	1.6°C/second	60°C	1 minute
3 (Dissociation)	0.075°C/second	95°C	15 seconds

^[1] For the 7500 Real-Time PCR System, use the default ramp rate.

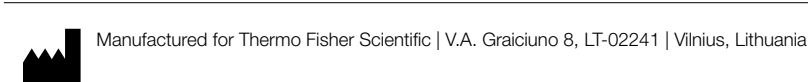
Note: A dissociation step must be performed immediately after the real-time PCR run with Fisherbrand™ SYBR™ Green qPCR Master Mix.

3. Set up the options.
 - Experiment type—Standard curve
 - Reagent—SYBR™ Green reagents
 - Reporter—SYBR™ Green
 - Quencher—None
 - Passive reference dye
 - For instruments compatible with ROX™ passive reference, select ROX™ dye.
 - For instruments not compatible with ROX™ passive reference, no selection is needed.
 - Ramp speed—Standard or fast
 - Melt curve ramp increment—Continuous
4. Set the reaction volume appropriate for the reaction plate.
5. Load the reaction plate into the real-time PCR instrument.
6. Start the run.

Analyze the results

- 1. View the amplification plots.
- 2. Determine the baseline and threshold cycles (C_q) for the amplification curves using the instrument software.
- 3. Check for nonspecific amplification using melt curves.
- 4. Perform relative or absolute quantitation.

Option	Description
Relative quantitation	The target is compared to an internal standard using the comparative C_q method.
Absolute quantitation	The C_q of the unknown samples is compared against a standard curve with known copy numbers.



Revision history: Pub. No. MAN0028448 A.0

Revision	Date	Description
A.0	28 September 2023	New document for the Fisherbrand™ SYBR™ Green qPCR Master Mix.

The information in this guide is subject to change without notice.

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