Thermo Scientific™ AmpSure™ Universal SYBR™ Green qPCR Master Mix

Catalog Number A66732

Pub. No. MAN1000090 Rev. B

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Thermo Scientific™ AmpSure™ Universal SYBR™ Green qPCR Master Mix User Guide* (Pub. No. MAN1000089). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Contents and storage

Thermo Scientific™ AmpSure™ Universal SYBR™ Green qPCR Master Mix

Cat. No.	Amount	Number of 20–µL reactions	Storage
A66732	5 mL	500	–30°C to –10°C

Guidelines

For detailed instructions and ordering information for additional products, see the *Thermo Scientific™ AmpSure™ Universal SYBR™ Green qPCR Master Mix User Guide* (Pub. No. MAN1000089).

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 Tm 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)
Thermo Scientific™ AmpSure™ Universal 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 gDNA. 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	
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 Thermo Scientific™ AmpSure™ Universal 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.

     Note: Bio-Rad™ and Roche™ real-time PCR systems detect ROX™ dye but do not apply ROX™-based passive reference normalization, even when ROX™ dye is included in the master mix..
   - 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.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN1000090 B

Revision	Date	Description
B	28 July 2026	• Name has been updated to Thermo Scientific™ AmpSure™ Universal SYBR™ Green qPCR Master Mix. • Minor edits were made for style and consistency. • Catalog number of the sample has been removed.
A00	11 April 2024	New document for the Thermo Scientific™ SYBR™ Green qPCR Master Mix.

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

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