PowerUp™ SYBR™ Green Master Mix

Universal 2X master mix for real-time PCR workflows

Catalog Numbers A25741, A25742, A25743, A25776, A25777, A25778, A25779, A25780, A25918

Pub. No. MAN0028468 Rev. C.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *PowerUp™ SYBR™ Green Master Mix User Guide* (Pub. No. MAN0013511). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference is intended as a benchtop reference for experienced users of PowerUp™ SYBR™ Green Master Mix. For detailed instructions, supplemental procedures, and troubleshooting, see the *PowerUp™ SYBR™ Green Master Mix User Guide* (Pub. No. MAN0013511).

Guidelines

Input DNA template requirements

Use 1–10 ng single-stranded cDNA or 10–100 ng gDNA per reaction.

IMPORTANT! In some cases, cDNA generated with the SuperScript™ IV VILO™ Master Mix is not compatible with the PowerUp™ SYBR™ Green Master Mix. It can result in a high background signal. To ensure consistent results across different samples and assays, we recommend a different reverse transcription kit.

For kits recommended for reverse transcription of RNA to cDNA, see *PowerUp™ SYBR™ Green Master Mix User Guide* (Pub. No. MAN0013511).

If you use SuperScript™ IV VILO™ Master Mix and your results do not show a high background signal with your assay and sample, you can continue to use the SuperScript™ IV VILO™ Master Mix and the PowerUp™ SYBR™ Green Master Mix.

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 <10 µL are not recommended.

NTC controls

No template control (NTC) reactions can be used to identify PCR contamination. NTC reactions contain all reaction components (PowerUp™ SYBR™ Green Master Mix, primers, water) except sample, and therefore should not return a C_t value.

Methods

Set up the PCR reactions

1. Prepare the appropriate number of reactions, plus 10% overage.

Component	Volume (10 µL/well)	Volume (20 µL/well)
PowerUp™ SYBR™ Green Master Mix (2X)	5 µL	10 µL
Forward and reverse primers[1]	Variable	Variable
DNA template + Nuclease-Free Water[2]	Variable	Variable
Total	**10 µL**	**20 µL**

[1] For optimal performance in Fast and Standard modes, use 300–800 nM for each primer.
[2] Use 1–10 ng cDNA or 10–100 ng gDNA for each reaction.

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

PCR can be performed on the reaction plate up to 72 hours after completing the setup, when stored at room temperature. Protect the reaction plate from light if the PCR is not started immediately after the reactions are set up.

Set up and run the real-time PCR instrument

1. Place the reaction plate in the real-time PCR instrument.
2. Set the thermal cycling conditions using the default PCR thermal cycling conditions specified in the following tables according to the instrument cycling parameters and melting temperatures of the specific primers.

   Note: Standard cycling conditions are recommended for genomic DNA templates. Use only standard cycling conditions for the 7900HT Real-Time PCR Instrument and the 7500 Real-Time PCR Instrument.

Table 1 Fast cycling mode (primer T_m ≥60°C)

Step	Temperature	Time	Cycles
UDG activation	50°C	2 minutes	1
Activation (Dual-Lock™ DNA polymerase)	95°C	2 minutes	1
Denature	95°C	1 second[1] or 3 seconds[2]	40
Anneal/extend	60°C	30 seconds	

[1] When using a QuantStudio™ Real-Time PCR System or a ViiA™ 7 Real-Time PCR System.
[2] When using a 7500 Fast Real-Time PCR System, 7900HT Fast Real-Time PCR Instrument, StepOnePlus™ Real-Time PCR System, or StepOne™ Real-Time PCR System.

Table 2 Standard cycling mode (primer T_m ≥60°C)

Step	Temperature	Time	Cycles
UDG activation	50°C	2 minutes	1
Activation (Dual-Lock™ DNA polymerase)	95°C	2 minutes	1
Denature	95°C	15 seconds	40
Anneal/extend	60°C	1 minute	

Table 3 Standard cycling mode (primer T_m <60°C)

Step	Temperature	Time	Cycles
UDG activation	50°C	2 minutes	1
Activation (Dual-Lock™ DNA polymerase)	95°C	2 minutes	1
Denature	95°C	15 seconds	40
Anneal	55–60°C[1]	15 seconds	
Extend	72°C	1 minute	

[1] Anneal temperature should be set to the melting point for your primers.

3. Set the instrument to perform a default dissociation step.

   A dissociation step can be performed up to 72 hours after the real-time PCR run if the plate is stored in the dark and up to 24 hours after the real-time PCR run if the plate is exposed to light.

Table 4 Dissociation curve conditions (melt curve stage)

Step	Ramp rate	Temperature	Time
1	1.6°C/second	95°C	15 seconds
2	1.6°C/second	60°C	1 minute
3[1]	0.15°C/second	95°C	15 seconds

[1] Dissociation

Use the following settings for Applied Biosystems™ instruments:

- Experiment type: Standard curve
- Reagent: SYBR™ Green reagents
- Reporter: SYBR™
- Quencher: None
- Passive reference dye: ROX™
- Ramp speed: Standard or fast (choose the same setting as in step 2)
- Melt curve ramp increment: Continuous

4. Set the reaction volume appropriate for the type of plate being used for your PCR reaction.

5. Start the run.

Analyze results

1. View the amplification plots.

2. Calculate the baseline and threshold cycles (C_t) for the amplification curves using the instrument software.

3. Check for nonspecific amplification using dissociation curves.

4. Perform relative or absolute quantitation.

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

Revision history: Pub. No. MAN0028468 C.0

Revision	Date	Description
C.0	5 December 2023	The instructions to set up the PCR reactions were corrected to indicate that the reaction plate can be stored at room temperature for up to 72 hours. The plate should be protected from light.
B.0	19 January 2023	Information was added about the compatibility of the SuperScript™ IV VILO™ Master Mix with the PowerUp™ SYBR™ Green Master Mix (“Input DNA template requirements” on page 1).
A.0	6 October 2022	New document for the PowerUp™ SYBR™ Green Master Mix. This document replaces 100031508, Rev. D, with the following edits: • Information was added to indicate that cDNA generated with the SuperScript™ IV VILO™ Master Mix is not compatible with the PowerUp™ SYBR™ Green Master Mix. • The instruments in the instructions to set up and run the real-time PCR instrument were updated.