PowerTrack™ SYBR™ Green Master Mix

Master mix with a two-dye tracking system for real-time PCR workflows

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *PowerTrack™ SYBR™ Green Master Mix User Guide* (Pub. No. MAN0018825). 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 PowerTrack™ SYBR™ Green Master Mix. For detailed instructions, supplemental procedures, and troubleshooting, refer to the *PowerTrack™ SYBR™ Green Master Mix User Guide* (Pub. No. MAN0018825).

Guidelines

Requirements for input DNA

Use 1–10 ng of cDNA or 10–100 ng of gDNA per reaction.

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 <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.

Methods

Set up the plate document or plate file

Configure the plate document or plate file.

See the appropriate instrument user guide for detailed instructions.

Prepare the reagents

- Thaw the master mix.
- Once the master mix is thawed, swirl it to mix thoroughly.
- Thaw the DNA samples and primers on ice, vortex to mix, then centrifuge briefly.
- Vortex the Yellow Sample Buffer prior to use.

Prepare the PCR reactions

Note: The Yellow Sample Buffer is optional for the real-time PCR.

The Yellow Sample Buffer is supplied at a 40X concentration. It is added to the DNA template. The concentration of Yellow Sample Buffer in the final PCR must be 1X. It is recommended that the DNA template is 10–20% of the volume of the final PCR.

1. *(Optional)* Add the Yellow Sample Buffer (40X) to the amount of DNA that is used in the PCR.

Final reaction volume	Amount of Yellow Sample Buffer
20 µL	0.5 µL
10 µL	0.25 µL

The Yellow Sample Buffer is diluted to 1X in the final reaction. See the following tables.

2. *(Optional)* Vortex, then centrifuge the DNA and Yellow Sample Buffer.

3. Combine the master mix, the primers, and nuclease-free water according to the following tables.

4. Combine the master mix, the primers, and nuclease-free water with the DNA and Yellow Sample Buffer according to the following tables.

Note: If the Yellow Sample Buffer is not used, add nuclease-free water to achieve the total PCR volume.

Table 1 20–µL reaction

Component	Stock concentration	Final concentration	Volume for 1 reaction (20–µL reaction)	Volume for 4 reactions with 10% overage (20–µL reaction)[1]
Yellow Sample Buffer and DNA (step 1)				
DNA[2]	5 ng/µL	0.5 ng/µL	2 µL[3]	8.8 µL
Yellow Sample Buffer	40X	1X	0.5 µL	2.2 µL
Master mix, primers, and nuclease-free water (step 3)				
PowerTrack™ SYBR™ Green Master Mix	2X	1X	10 µL	44.0 µL
Forward and reverse primers[4]	8,000 nM	400 nM	1 µL	4.4 µL
Nuclease-free water	—	—	6.5 µL	28.6 µL
Total PCR volume	**—**	**—**	**20 µL**	**88 µL**

[1] 10% overage is recommended for pipetting variations.
[2] Use 1–10ng of cDNA.
[3] Does not exceed 8.5 µL.
[4] The final primer concentration can vary from 300–800 nM. A final concentration of 400 nM is recommended for primers with a Tm of 55°C.

Table 2 10–µL reaction

Component	Stock concentration	Final concentration	Volume for 1 reaction (10–µL reaction)	Volume for 4 reactions with 10% overage (10–µL reaction)[1]
Yellow Sample Buffer and DNA (step 1)				
DNA[2]	5 ng/µL	0.5 ng/µL	1 µL[3]	4.4 µL
Yellow Sample Buffer	40X	1X	0.25 µL	1.1 µL
Master mix, primers, and nuclease-free water (step 3)				
PowerTrack™ SYBR™ Green Master Mix	2X	1X	5 µL	22.0 µL
Forward and reverse primers[4]	8,000 nM	400 nM	0.5 µL	2.2 µL
Nuclease-free water	—	—	3.25 µL	14.3 µL
Total PCR volume	**—**	**—**	**10 µL**	**44 µL**

[1] 10% overage is recommended for pipetting variations.
[2] Use 1–10ng of cDNA.
[3] Does not exceed 4.25 µL.
[4] The final primer concentration can vary from 300–800 nM. A final concentration of 400 nM is recommended for primers with a Tm of 55°C.

IMPORTANT! The reaction turns green due to the Yellow Sample Buffer added to the DNA and the inert blue dye in the master mix.

5. Mix the components thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.

6. Transfer the appropriate volume of each reaction to each well of an optical plate.

7. 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 8 hours after completing the set-up, when stored at room temperature protected from light.

Set up and run the real-time PCR instrument

1. Set up the thermal protocol according to one of the following tables.

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

   Table 3 Fast cycling mode

Step	Temperature	Duration	Cycles
Enzyme activation	95°C	2 minutes	1
Denature	95°C	5 seconds	40
Anneal/extend	60°C	30 seconds	

   Table 4 Standard cycling mode

Step	Temperature	Duration	Cycles
Enzyme activation	95°C	2 minutes	1
Denature	95°C	15 seconds	40
Anneal/extend	60°C	60 seconds	

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

   Table 5 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] Use the default ramp rate for the StepOnePlus™ Instrument.

   Table 6 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] Use the default ramp rate for the StepOnePlus™ Instrument.

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

3. Set up the options.
   - Experiment type: Standard curve
   - Reagent: SYBR™ Green reagents
   - Reporter: SYBR™ Green
   - Quencher: None
   - Passive reference dye: ROX™ dye
   - Ramp speed: Standard or fast
   - Melt curve ramp increment (all instruments, except StepOnePlus™ instrument): Continuous

(StepOnePlus™ only): Step and hold

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 either the standard curve or comparative C_q method.
Absolute quantitation	The C_q of the unknown samples is compared against a standard curve with known copy numbers.

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

Revision history: Pub. No. MAN0018826

Revision	Date	Description
B.0	29 July 2022	The volumes for preparing the PCR reactions were corrected (Table 1 on page 2 and Table 2 on page 2).
A.0	30 January 2020	New document.