By Royal Charter

Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 13485:2016 & EN ISO 13485:2016

This is to certify that:

Thermo Fisher Scientific Baltics UAB
V. A. Graiciuno 8
Vilnius
LT-02241
Lithuania

Holds Certificate Number: **MD 642790**

and operates a Quality Management System which complies with the requirements of ISO 13485:2016 & EN ISO 13485:2016 for the following scope:

Design, development, and manufacturing of reagents, proteins, nucleic acids, nucleotides, antibodies, associated kits, and materials intended for ex-vivo separation of human cells for in vitro diagnostics, for further manufacturing and applied market applications, including processes under aseptic condition.

For and on behalf of BSI:

Graeme Tunbridge, Senior Vice President Medical Devices

Original Registration Date: 2016-02-15
Effective Date: 2024-05-23
Latest Revision Date: 2024-04-18
Expiry Date: 2027-05-22

UKAS MANAGEMENT SYSTEMS 0003

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CERTIFICATE OF ANALYSIS

A46113 **PowerTrack™ SYBR™ Green Master Mix**

Packaging Lot: **3122947**

Expiry Date: 31.10.2026 (DD.MM.YYYY)

Storage: at -20±5°C in the dark

Note: **For Research Use Only. Not for use in diagnostic procedures.**

Filling lots for components in package:

Lot	Quantity	Description
3121856	50 mL	PowerTrack™ SYBR™ Green Master Mix
3115327	4 × 1.25 mL	40X Yellow Sample Buffer

QUALITY CONTROL

Parameter	Method	Requirement	Result
Functional Test	The product is functionally tested by qPCR analysis. It must demonstrate functional performance using a target concentration of plasmid DNA.	Average Ct for the target concentration is between 20 and 23	Pass
dNTP Concentrations	Determined by analytical method.	Within range of target concentration	Pass
Mg^{2+} Concentration	Determined by analytical method.	Within range of target concentration	Pass
K^{+} Concentration	Determined by analytical method.	Within range of target concentration	Pass
RNase Activity	Determined by analytical method.	No detectable activity level	Pass
DNase Activity	Determined by analytical method.	No detectable activity level	Pass
E. coli DNA Level	Determined by analytical method.	No detectable level	Pass

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė

PowerTrack SYBR Green Master Mix

Easy-to-use and flexible gene expression master mix for real-time PCR

Applied Biosystems™ PowerTrack™ SYBR Green Master Mix is a preformulated, optimized, universal 2X master mix for real-time PCR. Building on over 25 years of innovation and product excellence in qPCR, our PowerTrack SYBR Green Master Mix is designed for superior performance and ease of use with a two-color tracking dye system for the most common real-time PCR applications.

Features include:

- Built-in two-color tracking dye system where pipetting has occurred
- Broad primer T_m and primer concentration compatibility allows flexibility in qPCR reaction setup with minimal optimization
- Superior specificity and tight reproducibility in C_t values over a broad dynamic range improve data quality
- Compatible with Invitrogen™ SuperScript™ IV VILO™ Master Mix reverse transcription for fast, reproducible results
- Formulated with UNG and dUTP to prevent contamination of downstream reactions by carryover PCR products
- Broad instrument compatibility

Built-in visual indicator to aid in reaction setup

PowerTrack SYBR Green Master Mix is designed to provide ease in visualization of sample addition to the master mix. The master mix contains an inert blue dye and a separate, optional yellow sample buffer. The yellow sample buffer is added separately to indicate that sample has been added to the reaction, based on a visual color change of the reaction mix from blue to green. The benefit of using the tracking dye is to provide convenience via visualization of the color change chemistry and avoid errors that can occur due to pipetting mistakes. The yellow sample buffer is provided to aid in reaction setup for your own peace of mind but is not required to obtain superior results with PowerTrack SYBR Green Master Mix.

Formulated for maximum specificity and reproducibility

PowerTrack SYBR Green Master Mix uses an antibody-mediated hot-start mechanism to provide tight control over *Taq* enzyme activation and help prevent early activity of the polymerase at low temperatures that can lead to nonspecific amplification.

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High specificity

In an evaluation of 24 different primer sets used with PowerTrack SYBR Green Master Mix, a single melt curve was obtained in 100% of reactions. In contrast, nonspecific amplification was observed for some of the same targets with several master mixes from other suppliers, as shown by multiple peaks in the melt curves (Figure 1). Verification of primer specificity in SYBR Green reactions is essential to data quality and validity [1]. The high specificity enabled by PowerTrack SYBR Green Master Mix allows you to spend less time optimizing and redesigning primers to get high-quality data.

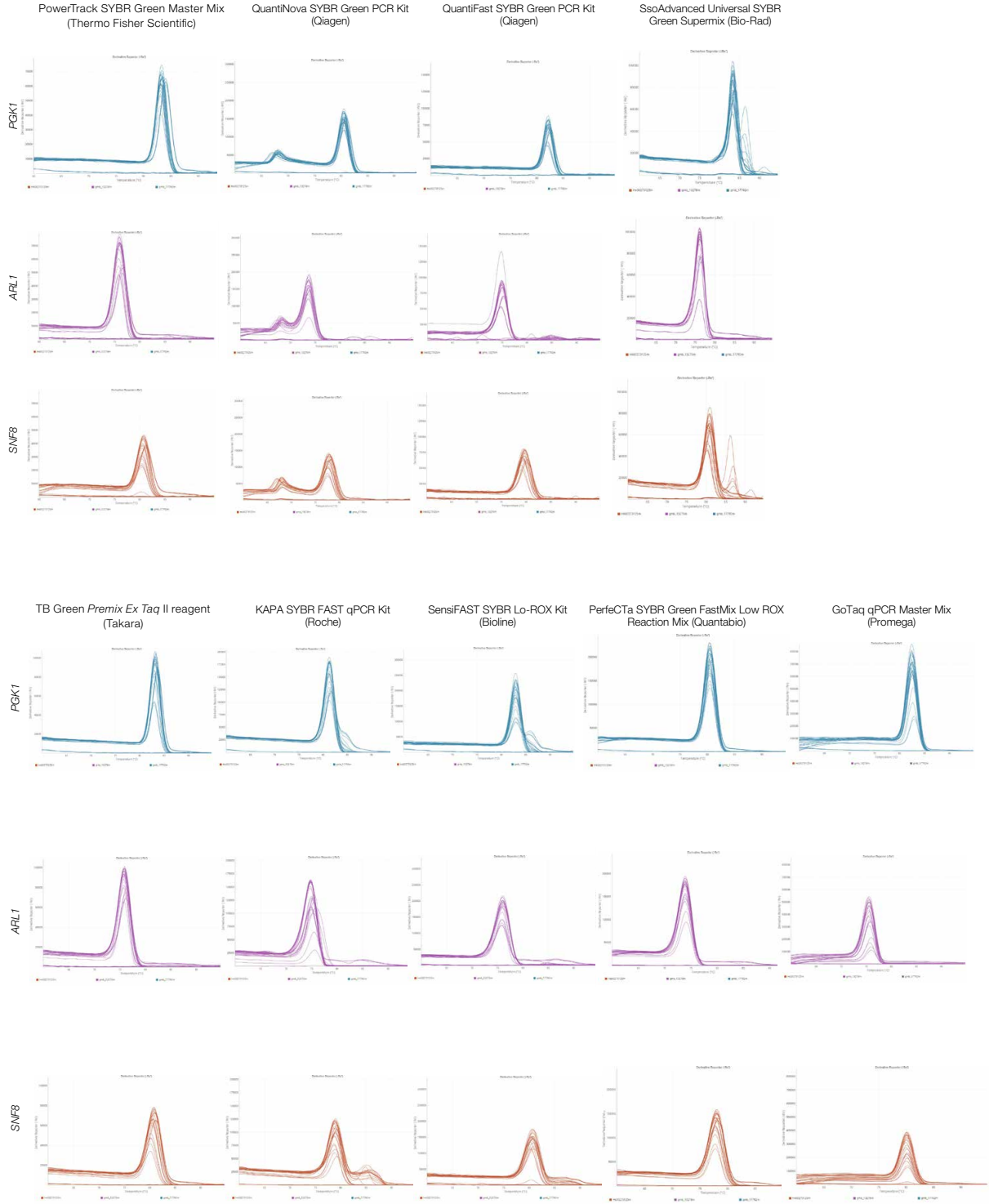

Figure 1. Target specificity. Real-time PCR was performed using universal human reference (UHR) cDNA and primers targeting *PGK1* (phosphoglycerate kinase 1), *ARL1* (ADP-ribosylation factor-like protein 1), and *SNF8* (vacuolar-sorting protein). Reactions (10 µL) were run in quadruplicate using the indicated master mixes on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System. Several master mixes from other suppliers show a second peak in the melt curve analysis attributed to amplification of nonspecific product.

PowerTrack SYBR Green Master Mix powers through traditionally difficult targets

Amplification curves were obtained for *PGK1* over a 6-log dilution series of UHR cDNA. PowerTrack SYBR Green Master Mix delivers accurate results over a wide dynamic range of concentrations as shown by tight curves between replicates and superior PCR efficiency (Figure 2).*

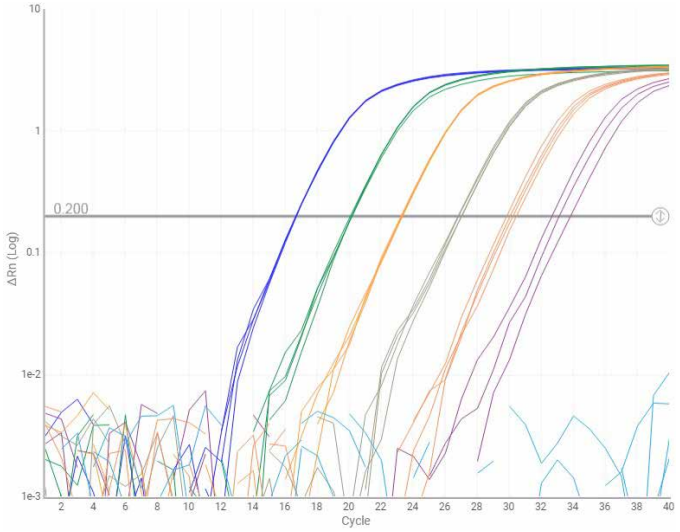

Figure 2. Linear dynamic range. PowerTrack SYBR Green Master Mix enables reliable results across a range of cDNA concentrations. Amplification curves were obtained for *PGK1* over a dilution series (10 ng to 100 fg and no template control (NTC)) of UHR cDNA. Reactions were run in quadruplicate on the QuantStudio 5 Real-Time PCR System using 60ºC as the annealing T_m for primers.

Excellent reproducibility

Reproducibility is another important measure of data quality in real-time PCR, and reproducibility is often affected at low template concentrations, where the effects of variability are exacerbated. However, PowerTrack SYBR Green Master Mix demonstrated excellent reproducibility over a wide dynamic range with a variety of targets and reverse transcription (RT) kits tested (Figure 3). Tighter reproducibility allows for greater statistical significance when analyzing low-abundance transcripts and smaller fold changes.

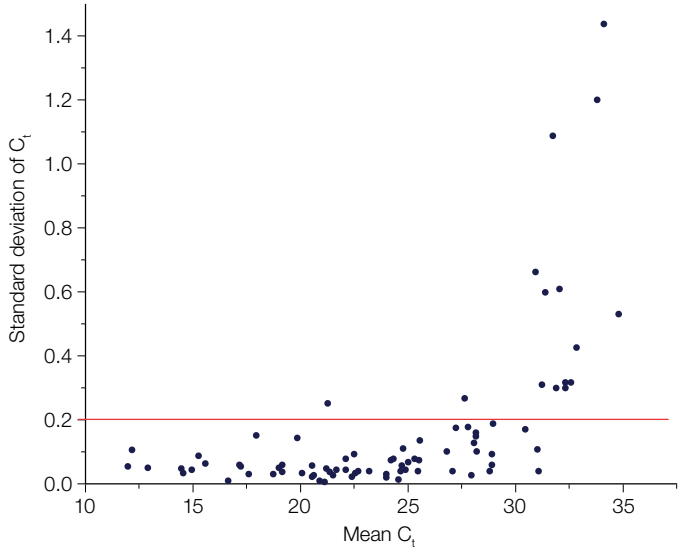

Figure 3. Reproducibility of data. PowerTrack SYBR Green Master Mix shows reproducibility over a wide dynamic range. Six assays (PGK1, ARL1, SNF8, DF, GAPDH, and Corf1 19) were run in quadruplicate with UHR cDNA generated from four different RT kits (SuperScript IV VILO Master Mix, Applied Biosystems™ High-Capacity RNA-to-cDNA Kit, iScript™ cDNA Synthesis Kit, and QuantiTect™ RT Kit) run with a 6-fold dilution series and 400 nM primer concentration. Assays were performed on the QuantStudio 5 Real-Time PCR System.

* Besides the master mix, other assay conditions and reagent concentrations may affect dynamic range; individual results may vary.

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Broad instrument compatibility

PowerTrack SYBR Green Master Mix can be used in either standard or fast cycling mode and is compatible with all Applied Biosystems™ real-time PCR instruments. It is also compatible with the Bio-Rad CFX96™, CFX384™, and iQ™5 instruments, as well as the Roche LightCycler™ 480 and Agilent Mx3005P™ instruments.

Why ROX dye matters

ROX™ dye is an inert reference dye used in RT-qPCR, often added to a master mix. It is effective in normalizing fluorescence across all samples. ROX dye removes fluorescence variations, such as those caused by bubbles in the reactions. Applied Biosystems™ master mixes contain a proprietary ROX dye, specifically formulated for a wide range of PCR instruments and for compatibility with a wide range of differing instrument light sources and filter sets. Most other manufacturers use a ROX dye that contains only a single excitation peak. These manufacturers may require a ROX dye to be spiked into the reaction at a concentration appropriate to the instrument. Alternatively, they may require selection of either a “low ROX” or “high ROX” master mix, depending on the concentration.

Heat-labile UNG for carryover contamination control

Contamination is a major concern in labs that routinely run PCR due to the potential for false-positive results. The inclusion of UNG and dUTP in the PowerTrack SYBR Green Master Mix allows any previously amplified PCR products to be degraded and helps prevent contamination of subsequent qPCR reactions.

Reference

1. Bustin SA, Benes V, Garson JA et al. (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* 55:611–622.

Ordering information

Product	Quantity	Cat. No.
PowerTrack SYBR Green Master Mix, Mini Pack (1 mL)	100 reactions	A46012
PowerTrack SYBR Green Master Mix, 1-Pack (1 x 5 mL)	500 reactions	A46109
PowerTrack SYBR Green Master Mix, 2-Pack (2 x 5 mL)	1,000 reactions	A46110
PowerTrack SYBR Green Master Mix, 5-Pack (5 x 5 mL)	2,500 reactions	A46111
PowerTrack SYBR Green Master Mix, 10-Pack (10 x 5 mL)	5,000 reactions	A46112
PowerTrack SYBR Green Master Mix, Bulk Pack (1 x 50 mL)	5,000 reactions	A46113

Find out more at **thermofisher.com/sybr**

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PowerTrack™ SYBR™ Green Master Mix

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

Catalog Numbers A46012, A46109, A46110, A46111, A46112, A46113

Pub. No. MAN0018826 Rev. B.0

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.