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Ion Library TaqMan™ Quantitation Kit

USER GUIDE

Catalog Number 4468802

Publication Number MAN0015802

Revision D.0

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Revision history: MAN0015802 D.0 (English)

Revision	Date	Description
D.0	22 November 2022	Updated manufacturer address to Vilnius.
C.0	27 July 2020	• Added cycling conditions specific to 7500 Fast Real-Time PCR Instrument, ≥300-bp libraries, and Ion AmpliSeq™ Exome RDY libraries in "Cycling conditions" on page 8. • Updated serial dilution example in "Prepare serial dilutions" on page 10.
B.0	30 November 2017	• Update to component name • Correction to 7500 Fast Real-Time PCR Instrument cycling conditions
A.0	20 October 2016	Rebranding and chapter reorganization

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

Contents

1 Product information

Product description

The Ion Library TaqMan™ Quantitation Kit contains reagents for quantitative real-time PCR (qPCR) for the quantification of unamplified and amplified libraries prepared using Ion library kits. Ion libraries are quantified before template amplification onto Ion Sphere™ Particles (ISPs) and sequencing on the Ion PGM™, Ion Proton™, Ion S5™, and Ion S5™ XL Systems.

Key features of the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802) include:

- AmpliTaq™ DNA Polymerase UP, which is a recombinant Taq DNA polymerase that is activated using a proprietary hot-start mechanism that blocks polymerase activity at ambient temperatures. The enzyme is activated after the initial denaturation step in PCR cycling, preventing early nonspecific amplification products from being generated, which results in increased sensitivity, specificity, and yield.
- Uracil DNA glycosylase (UDG): UDG and dUTP in the qPCR mix prevent the reamplification of carryover products between qPCR reactions.

See **www.thermofisher.com** for further information about methods and kits for preparation of Ion libraries.

Dye characteristics

Reference	Reporter	Quencher
ROX™	FAM™	MGB

Library compatibility

The Ion Library TaqMan™ Quantitation Kit can be used to quantify all Ion libraries except:

- Libraries prepared as described in the *Ion Amplicon Library Preparation (Fusion Method) User Guide* (Pub. No. 4468326) are not compatible with this kit. Libraries prepared using trP1 and A Adapter sequences are incompatible with this qPCR method.
- Libraries prepared with the Ion Total RNA-Seq Kit v2 (Cat. No. 4475936 and 4479789) are not compatible with this kit. Libraries prepared using the A and P1 adapters supplied in the Ion Total RNA-Seq Kit v2 are incompatible with this qPCR method.

Instrument compatibility

The Ion Library TaqMan™ Quantitation Kit can be used with a wide range of real-time instruments, including the following Applied Biosystems™ instruments.

- ViiA™ 7
- QuantStudio™ 3
- QuantStudio™ 5
- QuantStudio™ 6
- QuantStudio™ 7
- QuantStudio™ 12 K
- StepOne™
- StepOnePlus™
- 7500 Fast
- 7900 HT
- 7900 HT Fast

Contents and storage

Contents	Amount	Storage[1]
Ion Library qPCR Master Mix	2 × 1.25 mL	–25°C to –15°C [2]
Ion Library TaqMan™ Quantitation Assay, 20X	250 µL	–25°C to –15°C
E. coli DH10B Control Library	2 × 25 µL	

[1] Minimize freeze-thaws.
[2] May be stored at 2–8°C for up to one year.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Real-time PCR instrument: see “Instrument compatibility” on page 5 for a list of Applied Biosystems™ real-time PCR instruments[1].	Various
PCR plates appropriate for your instrument, for example: MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4346906
Optical sealer for the PCR plate, for example: MicroAmp™ Optical Adhesive Film	4360954
Nuclease-Free Water (not DEPC-Treated)	AM9930
Nonstick, RNase-Free Microcentrifuge Tubes, 0.5 mL	AM12350
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450
Microcentrifuge	**MLS**
Picofuge	**MLS**
Vortex mixer	**MLS**
Pipettors: 20 µL, 200 µL, 1000 µL	**MLS**
Barrier (filter) pipettor tips	**MLS**

[1] Also compatible with instruments from other suppliers

Workflow

The workflow is illustrated in Figure 1. The *E. coli* DH10B Control Library is diluted serially to generate a set of standards. The Ion sample library is diluted to a concentration that falls within the range of the Control Library standards. Following qPCR of both the Control Library standards and the diluted Ion sample library, a standard curve is generated, from which the concentration of the Ion sample library is calculated.

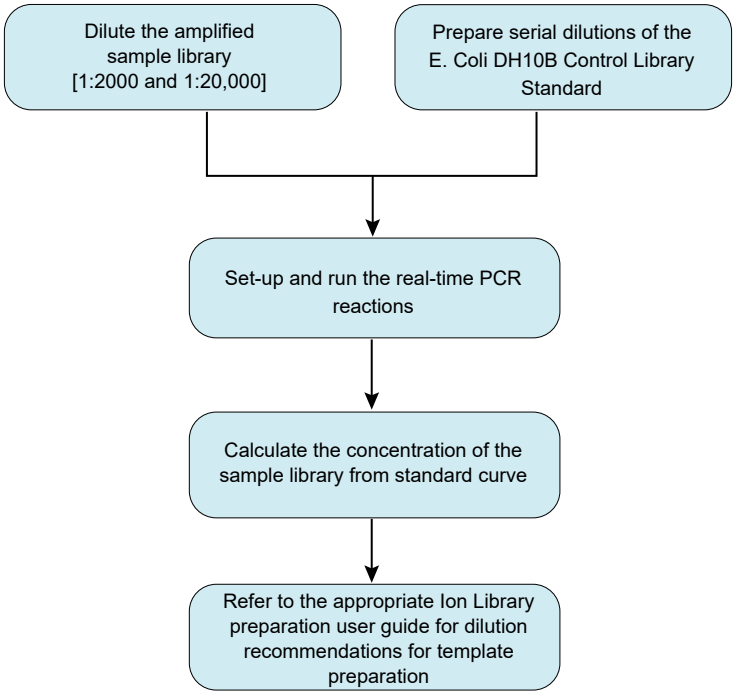

Figure 1 Library quantification with the Ion Library TaqMan™ Quantitation Kit

2 Methods

Procedural guidelines

Before you begin

- Thaw frozen components on ice.

Guidelines for reaction setup

- Maintain a sterile environment when handling Ion libraries and the *E. coli* DH10B Control Library, to avoid contamination from DNases.
- Use non-stick microfuge tubes for the serial dilution of the *E. coli* DH10B Control Library for best results.
- Make sure all equipment that comes into contact with DNA is sterile, including pipette tips and microcentrifuge tubes.
- qPCR reaction volumes can be scaled from 5 µL to 100 µL, depending on the plate size and instrument (for example, the 7500 Fast Real-Time PCR System uses 20 µL per well in both standard and fast mode).
- All samples, including no-template controls (NTC), should be set up in triplicate to increase accuracy.
- For 384-well plates, we recommend a maximum reaction volume of 10 µL per well.

Cycling conditions

Thermal cycling conditions for compatible Applied Biosystems™ real-time PCR instruments are presented in the following tables. For a list of compatible instruments, see “Instrument compatibility” on page 5. Use these conditions as a starting point for this kit.

- For cycling conditions compatible with all instruments except 7500 Fast Real-Time PCR Instrument, see Table 1.
- For cycling conditions compatible with the 7500 Fast Real-Time PCR Instrument, see Table 2.
- For cycling conditions compatible with ≥300-bp libraries and Ion AmpliSeq™ Exome RDY libraries, see Table 3.

Table 1 Cycling conditions compatible with all instruments *except* 7500 Fast Real-Time PCR Instrument

Stage	Temp	Time
Hold (UDG incubation)[1]	50°C	2 min
Hold (polymerase activation)[2]	95°C	20 sec
Cycle (40 cycles)	95°C	1 sec
	60°C	20 sec

[1] Required for optimal UDG activity.

[2] Required for AmpliTaq™ DNA Polymerase activation and template denaturation.

Table 2 Cycling conditions compatible with the 7500 Fast Real-Time PCR Instrument

Stage	Temp	Time
Hold (UDG incubation)[1]	50°C	2 min
Hold (polymerase activation)[2]	95°C	20 sec
Cycle (40 cycles)	95°C	3 sec
	60°C	30 sec

[1] Required for optimal UDG activity.

[2] Required for AmpliTaq™ DNA Polymerase activation and template denaturation.

Table 3 Cycling conditions compatible with ≥300-bp libraries and Ion AmpliSeq™ Exome RDY libraries

Stage	Temp	Time
Hold (UDG incubation)[1]	50°C	2 min
Hold (polymerase activation)[2]	95°C	2 min
Cycle (40 cycles)	95°C	15 sec
	60°C	1 min

[1] Required for optimal UDG activity.

[2] Required for AmpliTaq™ DNA Polymerase activation and template denaturation.

Prepare serial dilutions

Keep all dilutions on ice.

1. Prepare serial dilutions of the ***E. coli* DH10B Control Library**.
   a. Prepare five sequential 10-fold dilutions in non-stick microcentrifuge tubes from the Control Library (~68 pM stock concentration) in Nuclease-free Water, as shown in the following table.

   Note: Prepare sufficient volume of each dilution for the size of your qPCR reactions and the number of replicates. For example, for a 20-µL qPCR reaction volume, prepare 5 µL of diluted standard per reaction, or 15 µL per triplicate plus a 5-10% overage for pipetting loss.

   Example of a 50-µL dilution of the ***E. coli* DH10B Control Library**

Standard	Library input	Water[1]	Fold dilution	Concentration
1	5 µL (undiluted)	45 µL	0.1	6.8 pM
2	5 µL Std 1	45 µL	0.01	0.68 pM
3	5 µL Std 2	45 µL	0.001	0.068 pM
4	5 µL Std 3	45 µL	0.0001	0.0068 pM
5	5 µL Std 4	45 µL	0.00001	0.00068 pM

[1] Nuclease-free Water (not DEPC-treated)

2. Prepare serial dilutions of the **sample library.**
   a. Prepare three sequential dilutions of the sample library, as shown in the following table.

   Note: Prepare dilutions of the sample library that target a concentration within the range of the serial dilutions of the control library.

   Prepare sufficient volume of each dilution for the size of your qPCR reactions and the number of replicates. For example, for a 20-µL qPCR reaction volume, prepare 5 µL of diluted standard per reaction, or 15 µL per triplicate plus a 5-10% overage for pipetting loss.

   Example dilution of the **sample library**

Sample	Library input	Water[1]	Dilution
1	5 µL (undiluted)	95 µL	1:20
2	5 µL of sample 1	195 µL	1:800
3	5 µL of sample 2	120 µL	1:20,000

[1] Nuclease-free Water (not DEPC-treated)

3. Mix the diluted solutions by gently pipetting up and down at least 10 times.

IMPORTANT! Do not vortex.

Set up and run qPCR reactions

1. Gently but thoroughly mix each component.
2. Centrifuge briefly to bring the contents to the bottom of the tube.

   IMPORTANT! Do not vortex the Ion Library TaqMan™ qPCR Mix.

3. Prepare a master mix of components on ice, as described in the following table.

   Scale the volumes based on the number and volume of your qPCR reactions. At a minimum, prepare sufficient volume for three technical replicates of each control dilution, library dilution, and no-template control.

Component	Volume per 20-µL reaction
Ion Library TaqMan™ qPCR Mix, 2X	10 µL
Ion Library TaqMan™ Quantitation Assay, 20X	1 µL
Nuclease-free Water	to 15 µL

4. For each reaction, pipette 15 µL of the master mix into a well of the PCR plate.
5. Add 5 µL of library (prepared in "Prepare serial dilutions" on page 10) or Nuclease-free Water to each well.
   - Control wells: appropriate dilutions of *E. coli* DH10B Control Library
   - Sample wells: appropriate dilutions of sample library
   - No-template control (NTC) wells: Nuclease-free Water
6. Seal the plate, then centrifuge the plate briefly to collect contents at the bottom of the wells and eliminate air bubbles.
7. Place the plate in the real-time qPCR instrument, run the reactions, then collect the real-time data.

   Note: Follow the manufacturer's instructions for your real-time qPCR instrument. Visit **www.thermofisher.com** for user guides for Applied Biosystems™ instruments.

Determine the concentration for the sample library

The instrument software calculates the diluted library concentration. The undiluted library concentration is calculated by multiplying the concentration determined with qPCR by the sample dilution (for example, 2,000 or 20,000).

1. Choose the C_t data from the appropriate library dilution.
   - For many libraries, more than one dilution of the sample library will fall within the standard curve. Use the C_t values from the most concentrated sample dilution to calculate the library concentration.
   - For very concentrated or very dilute libraries, use the C_t values from the dilution that falls within the standard curve.

2. Calculate the undiluted sample library concentration.

   Undiluted Library Concentration = (Concentration determined by qPCR) × (Library Dilution)

   Example: The diluted library concentration determined by qPCR is 6.11 pM for a 1:2,000 library dilution. See “Example standard curve” on page 13.

   Undiluted Library Concentration = (6.11 pM) × (2,000) = 12,200 pM (12.2 nM)

3. See the appropriate Ion template preparation user guide for dilution recommendations for template preparation. The Ion library and template preparation user guides can be found at **www.thermofisher.com**.

Example data

In the following examples, dilutions of the *E. coli* DH10B Control Library and multiple Ion libraries were prepared in triplicate. Real-time PCR reactions were run on the Applied Biosystems™ StepOnePlus™ Real-Time PCR System using the reagents supplied in this kit. In the amplification plot illustrated in Figure 2, the Control Library reactions appear in red (Std).

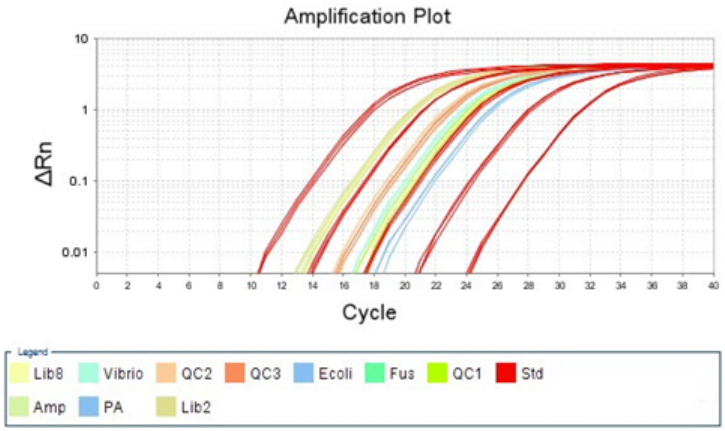

Figure 2 Example amplification plot

Example standard curve

The graph illustrates a typical standard curve generated from serial dilutions of the *E. coli* DH10B Control Library (red data points). The dynamic range of the qPCR assay is typically 10^4. The concentrations of the Control Library standards are plotted on the X-axis. The real-time qPCR instrument software calculates the concentration of the diluted sample library compared to the Control Library, as shown in the inset.

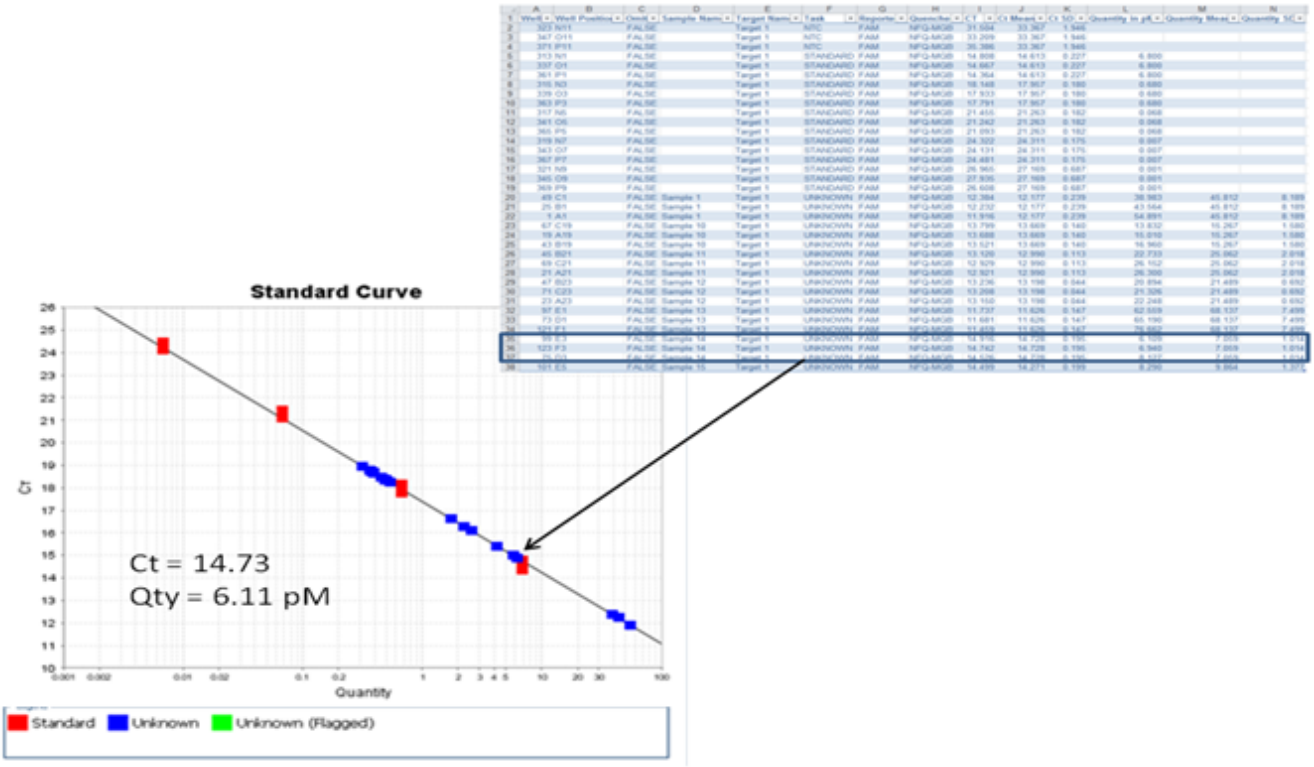

Figure 3 Example standard curve

A Troubleshooting

Observation	Possible cause	Recommended action
Signal appears in the no-template controls (NTCs)	The library or reagents were contaminated by nucleic acids (DNA, cDNA).	Some spurious amplification may occur in NTCs at high C_ts (for example, ~30). This is above the C_t range for actual template, and thus has no effect on quantitation. Take standard precautions to avoid contamination when preparing your PCR reactions. Ideally, amplification reactions should be assembled in a DNA-free environment. We recommend using aerosol-resistant barrier tips.
No PCR product is evident, either on the qPCR graph or on a gel	The protocol was not followed correctly.	Verify that all steps have been followed and the correct reagents, dilutions, volumes, and cycling parameters have been used.
	The library contained inhibitors, nucleases or proteases, or was otherwise degraded.	Purify or repurify the library.
PCR product is evident on a gel, but not in the qPCR graph	qPCR instrument settings were incorrect.	Confirm that you are using the correct instrument settings (dye selection, reference dye, filters, and acquisition points).
	There was an issue with your specific qPCR instrument.	See your instrument manual for tips and troubleshooting.
C_t values for both library dilutions fall into the baseline of the amplification plot	The concentrations of the library dilutions were too high.	Set the baseline manually.
		Dilute the library > 1:20,000, then repeat the qPCR.
PCR efficiency is above 110%	The library contained inhibitors, nucleases, or proteases, or was otherwise degraded.	Purify or repurify the library. Inhibitors in the library may result in changes in PCR efficiency between dilutions.
	Nonspecific products were amplified.	Run the PCR products on a 4% agarose gel after the reaction to identify contaminants.
PCR efficiency is below 90%	The PCR conditions were suboptimal.	Verify that the reagents have not been freeze-thawed multiple times and have not remained at room temperature for too long. Verify that the amount of assay in the reaction is correct.

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/publications/i/item/9789240011311

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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