

USER GUIDE

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TaqMan® OpenArray® Pathway Panels

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About This Guide

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Revision history

Revision	Date	Description
A.00	October 2013	New document

Purpose of the guide

The *TaqMan[®] OpenArray[®] Pathway Panels User Guide* provides:

- general product information on the different types of TaqMan[®] OpenArray[®] Pathway Panels available.
- guidelines on how to select panels and inputs.
- procedures for Standard and Low Sample Input protocols.



Product information

Product information

Each of the six fixed-content gene expression TaqMan® OpenArray® Pathway Panels enables the identification of differentially expressed genes in key pathways through expression profiling and high-throughput analysis of commonly studied gene families involved in specific disease states, biological processes, and cellular regulation pathways (Table 1).

Pathway Panels overview

Each TaqMan® OpenArray® Pathway Panel contains between 597 and 828 genes per panel (Table 1). Four samples can be tested simultaneously on a single Pathway Panel and three samples on a Kinome Panel.

Table 1 TaqMan® OpenArray® Pathway Panels

Panels	Markers profile	Format	# per panel			Cat. no.	
			Samples	Assays	Controls	OpenArray® system	QuantStudio™ 12K Flex system
Human Kinome	Genes involved in regulation of cellular pathways	896	Up to 3	828	56	4475368	4475388
Human Stem Cell	Markers of embryonic stem cells (hESC)	672	Up to 4	631	22	4475370	4475390
Human Cancer	Genes involved in key pathways of cancer	672	Up to 4	648	24	4475371	4475391
Human Signal Transduction	Genes involved in key signaling pathways	672	Up to 4	597	24	4475372	4475392

Panels	Markers profile	Format	# per panel			Cat. no.	
			Samples	Assays	Controls	OpenArray® system	QuantStudio™ 12K Flex system
Human Inflammation	Markers of pathways of inflammation response	672	Up to 4	607	24	4475369	4475389
Mouse Inflammation	Markers of pathways of inflammation response	672	Up to 4	648	16	4475373	4475393

Pathway Panels topography

OpenArray® Pathway Panels are presented as 63-mm × 19-mm mid-density reaction plates. There are 3072 reaction through-holes in the plate; individual through-holes can accommodate a 33-nL reaction volume. Each plate contains 48 subarrays, each with 64 through-holes arranged in 8 × 8 grids. Hydrophilic and hydrophobic coatings allow reagents to be held within the through-holes (Figure 1). TaqMan® assays are pre-spotted into each through-hole and dried-down before shipping out to customers.

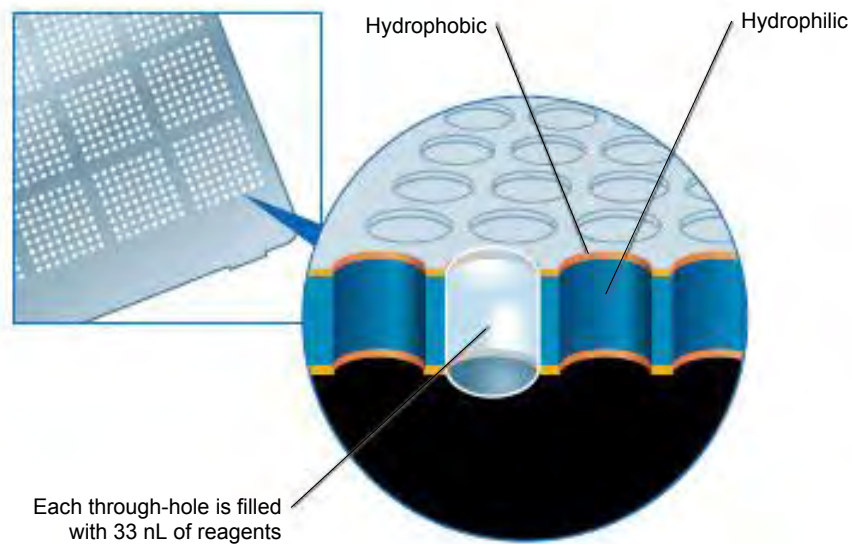


Figure 1 TaqMan® OpenArray® plate layout

For the 672-format Pathway Panels, the assays occupy 12 subarrays: A1-D3, A4-D6, A7-D9, and A10-D12 (Figure 2). This layout allows for loading up to four individual samples onto one single OpenArray® plate.

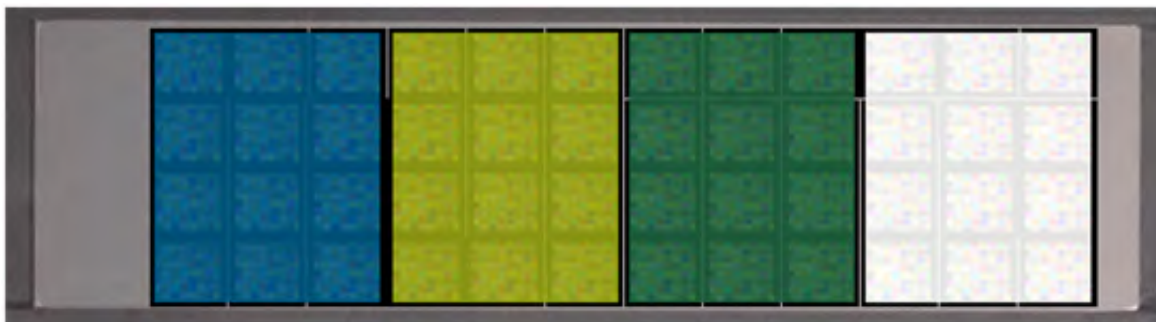


Figure 2 Layout example for the 672-format TaqMan® OpenArray® plate

The TaqMan® OpenArray® Kinome Pathway Panel features 828 assays in a 896-format, allowing for loading up to 3 samples onto one single TaqMan® OpenArray® plate. In the 896-format, the assays occupy 16 subarrays: A1-D4, A5-D8, and A9-D12 (Figure 3).

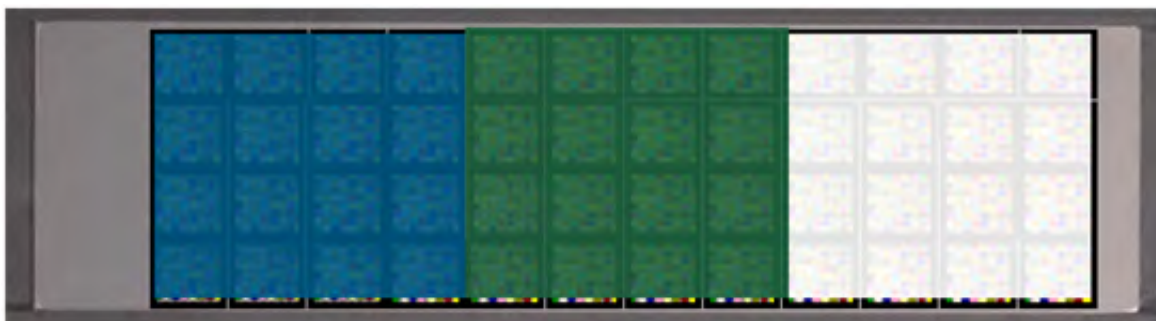


Figure 3 Layout example for the 896-format TaqMan® OpenArray® plate

Pathway Panels protocols overview

Table 2 Overview of the Standard and Low Sample Input protocols

	Standard ^[1]	Low Sample Input ^[2]			
Recommended RNA input	200 ng/μL	50-200 ng/μL	10-50 ng/μL	1-10 ng/μL	< 1 ng/μL
Minimum Volume of RNA for reverse transcription (RT) reactions	10 μL ^[3]	10 μL			
Total quantity of RNA for RT reactions	2 μg	500 ng-2 μg	100-500 ng	10-100 ng	< 10 ng
Final volume of RT reactions	20 μL ^[3]	20 μL			
Gene-specific RT ^[4]	No	No	Yes	Yes	Yes
Volume of RT reactions to use in pre-amplification reactions (per PreAmp pool) ^[5]	—	5 μL		10 μL	
Final volume of pre-amplification reactions (per PreAmp pool) ^[5]	—	20 μL		40 μL	
Pre-amplification cycles	—	12	14	16	18
Dilution after pre-amplification (after pools mixing)	—	1:20			
PCR reaction dilution	1:4	1:2			
Final dilution	1:8	1:80			

^[1] Chapter 2, "Standard protocol"

^[2] Chapter 3, "Low Sample Input protocol"

^[3] The RT volume for the Kinome panel is 24 μL with a minimum volume of RNA of 12 μL.

^[4] Appendix A, "Gene-specific reverse transcriptions (optional)"

^[5] Table 4

Required materials not supplied

The tables below list materials that are required to perform the different steps of the reactions but that are not provided with the panels. For more information on how to use each individual material, please consult the manufacturer's instructions.

Table 3 Required materials for reverse transcription reactions

Item	Cat. no.
SuperScript® VIL0™ cDNA Synthesis Kit	11754-050
RNase-free water	AM9935
Total RNA	Supplied by user
GeneAmp® PCR System 9700 Thermal Cycler	4314878
MicroAmp® Optical 96-well Reaction Plate (if preparing multiple samples)	N8010560
MicroAmp® Optical Adhesive Film	4360954

Table 4 Required materials for pre-amplification (needed only if using the Low Sample Input workflow)

Item	Cat. no.
TaqMan® PreAmp Master Mix	4391128
TaqMan® pooled gene-specific primers	Contact Technical Support for more information
cDNA	Supplied by user

Table 5 Required materials for PCR

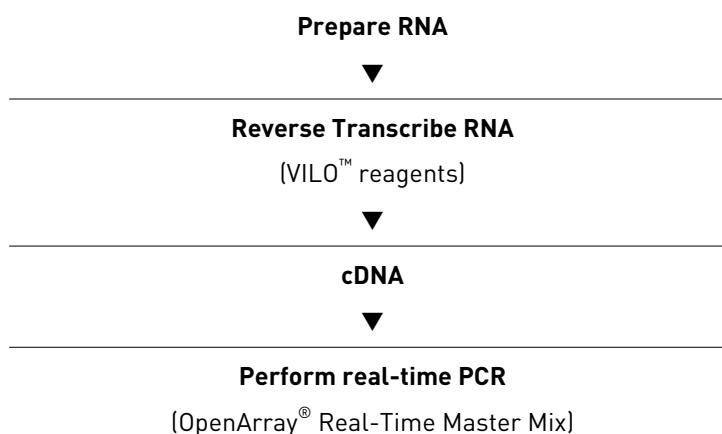
Item	Cat. no.
Reaction tubes, containing prepared cDNA samples	Supplied by user
2X TaqMan® OpenArray® Real-Time PCR Master Mix	4462164 (5 mL)
	or 4462159 (1.5 mL)
OpenArray® 384-Well Sample Plates	4406947
	or 4453929 (barcoded)
OpenArray® AccuFill™ System Tips	4457246
	or 4458107 (10 packs)
QuantStudio™ 12K Flex OpenArray® Accessories Kit	4469576



Standard protocol

Overview of the standard workflow

The following figure illustrates the workflow for using the TaqMan® OpenArray® Pathway Panels with 200 ng/μL of total RNA as starting material.



Quantity of starting material

We recommend using 10 μL of total RNA at a concentration of 200 ng/nL (2 μg total) for the Pathway Panels with 4 samples/plate (672-format) and 12 μL of total RNA at a concentration of 200 ng/nL (2.4 μg total) for the Kinome Panel with 3 samples/plate (896-format).

Quality of the starting RNA

We recommend that the starting RNA used with the Pathway Panels:

- Is extracted from raw materials of interest using an optimized protocol
- Does not contain inhibitors such as hemoglobin, heparin, and melanin
- Has an A_{260}/A_{280} ratio between 1.8 and 2.1
- Is intact as visualized by gel electrophoresis
- Has an RNA Integrity Number (RIN) between 6.5 and 10

Perform the reverse transcription

1. Thaw the SuperScript® VILO™ cDNA Synthesis Kit components and the RNA sample on ice.
2. Prepare the RT master mix by combining the following components for the number of reactions required.

Component	Volume ^[1] Pathways Panels (672-format)	Volume ^[1] Kinome Panel (896-format)
5X VILO™ Reaction Mix	4.0 µL	4.8 µL
10X SuperScript® Enzyme Mix	2.0 µL	2.4 µL
RNase-free water	4.0 µL	4.8 µL
RT master mix final volume	10.0 µL	12.0 µL

^[1] Add 10% overage to account for pipetting error.

3. Add 10 µL (for the Pathway Panels) or 12 µL (for the Kinome Panel) of sample RNA to the RT master mix.
4. Incubate at 25°C for 10 minutes.
5. Incubate at 42°C for 60 minutes.
6. Incubate at 85°C for 5 minutes, place on ice for 5 minutes, then spin down the contents of the microtube.

STOPPING POINT If needed, the obtained cDNA can be stored at –20°C for 2 months.

Perform the PCR

We recommend that the samples are prepared in centrifuge tubes or tube strips before they are pipetted into a 384-well sample plate (Table 5).

1. Mix the 2X TaqMan® OpenArray® Real-Time Master Mix (Table 5) by gently inverting the tube 10 times.
2. Prepare the PCR master mix by combining the following components for 1 OpenArray® plate.

Component	Volume ^[1]
2X TaqMan® OpenArray® Real-Time Master Mix	145.2 µL
RNase-free water	75.5 µL
PCR master mix final volume	220.7 µL

^[1] 10% overage is already included in the volumes above to account for pipetting errors.

- Mix the contents thoroughly by pipetting up and down.
- Thaw the cDNA samples at room temperature. Mix the samples by vortexing, then centrifuge for 1 minute at 1000 rpm.
- Distribute the PCR master mix in 4 tubes for pathway panels or 3 tubes for kinome panels and add the cDNA sample, according to the following table.

Component	Volume ^[1]	
	Pathway Panels (12 subarrays)	Kinome Panel (16 subarrays)
PCR master mix	50.2 μ L	66.9 μ L
cDNA	15.8 μ L	21.1 μ L
Total reaction volume	66 μ L	88.0 μ L

^[1] 10% overage is already included in the volumes above to account for pipetting errors.

- Mix the contents thoroughly by pipetting up and down 10 times.
- Distribute 5 μ L of PCR reactions into each well of the 384-well sample plate using a single- or multi-channel pipette. The contents of one well of a 384-well sample will load one subarray on the OpenArray[®] plate (Figure 4).

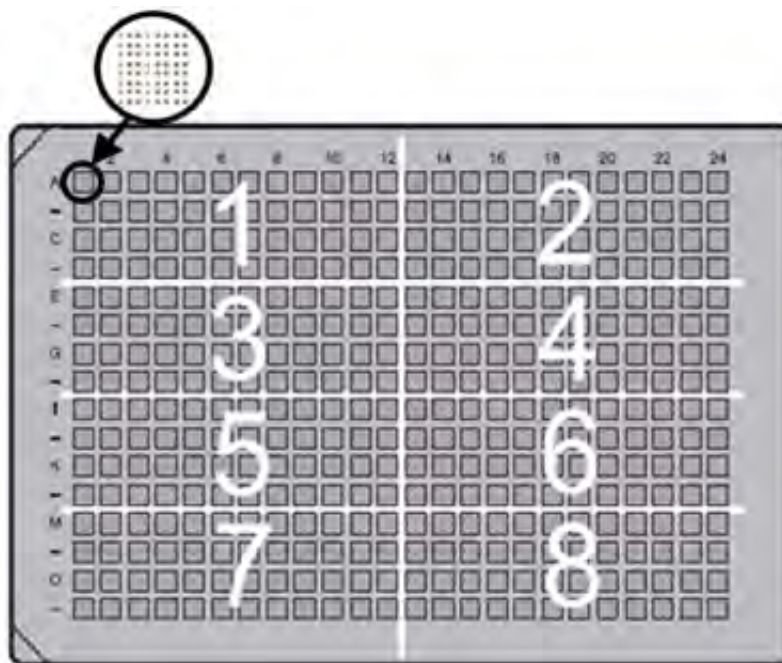


Figure 4 384-well sample plate layout

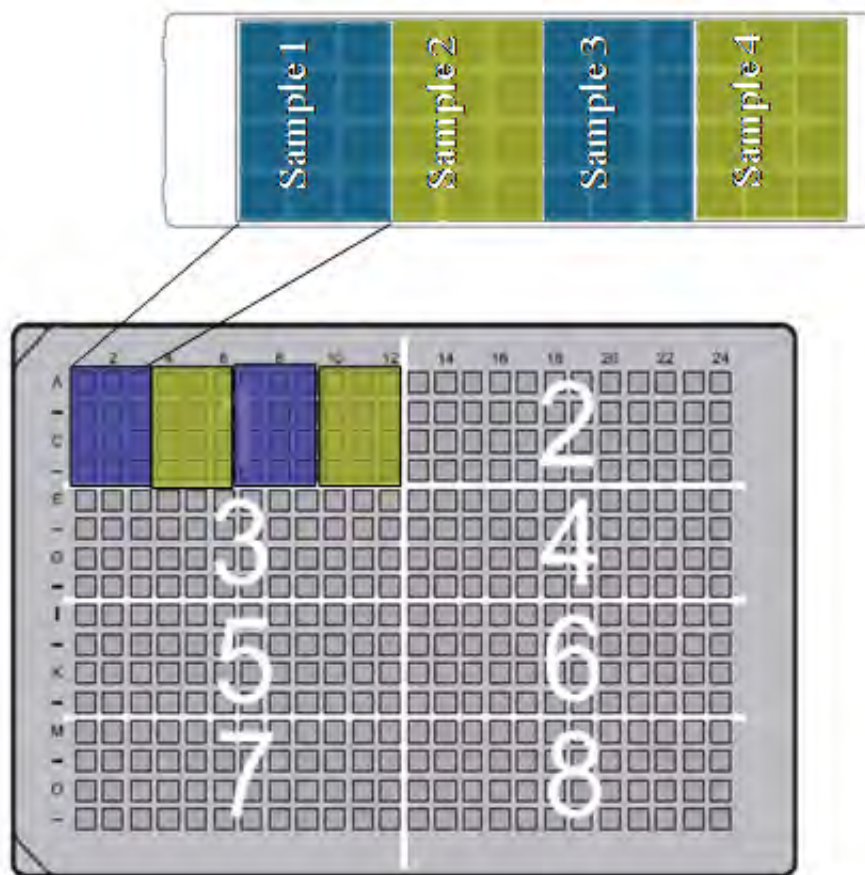


Figure 5 Loading schematic for the 672-format plate layout

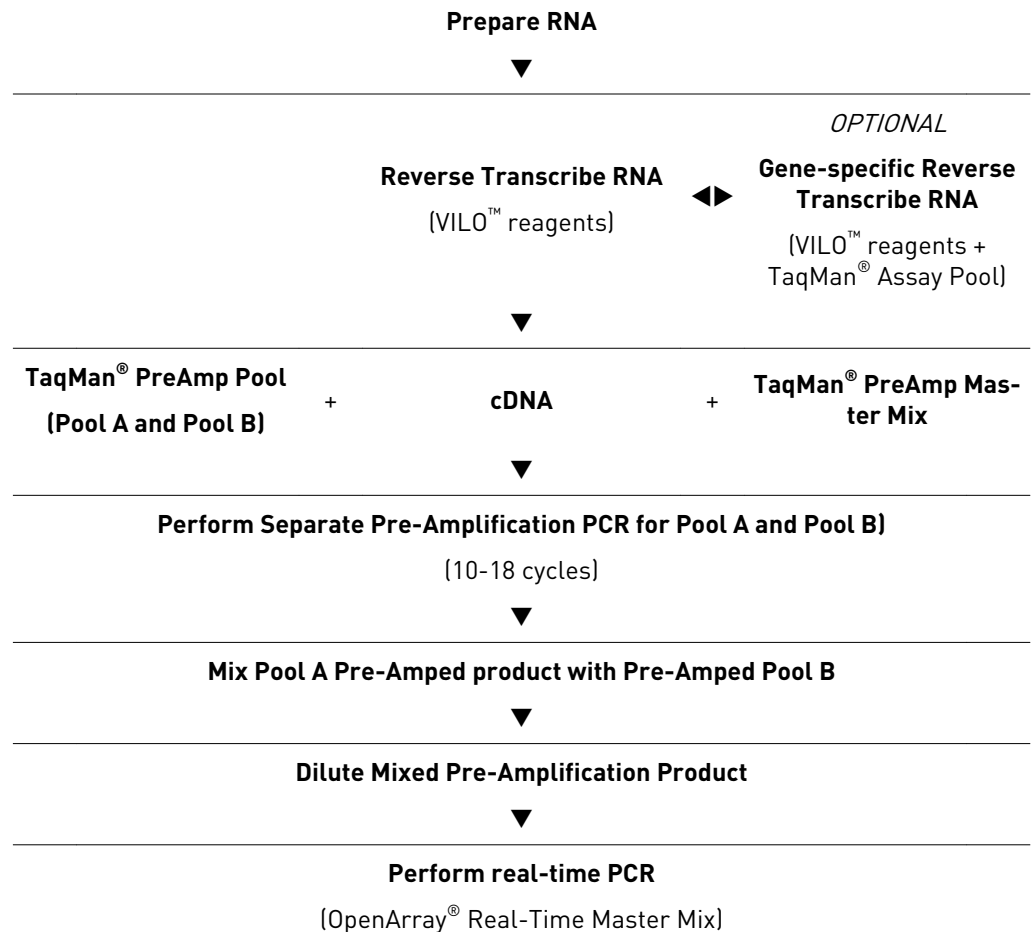
- To load 12 subarrays (672-format), distribute sample 1 into wells A1 through D3, sample 2 into wells A4 through D6, sample 3 into wells A7 through D9, and sample 4 into wells A10 through D12 (Figure 5).
 - To load 16 subarrays (Kinome Panel), distribute sample 1 into wells A1 through D4, sample 2 into wells A5 through D8, and sample 3 into A9 through D12.
8. Cover the plate with sealing tape.
 9. Centrifuge the sample plate for 1 minute at 1000 rpm to eliminate bubbles, then place the sample plate on ice.
 10. Load the samples with the master mix from the 384-well sample plate onto the OpenArray® Plate using the OpenArray® AccuFill™ System. For more information, consult *QuantStudio™ 12K Flex Real-Time PCR System OpenArray® Experiments Getting Started Guide* (Pub. no. 4470935).
 11. Run the PCR on the QuantStudio™ 12K Flex Real-Time PCR System using the appropriate .edt or .tpf file containing all the information pertaining to the assays. For more information, consult *QuantStudio™ 12K Flex Real-Time PCR System OpenArray® Experiments Getting Started Guide* (Pub. no. 4470935).

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Low Sample Input protocol

Overview of the Low Sample Input workflow

The following figure illustrates the workflow for using the TaqMan® OpenArray® Pathway Panels with less than 200 ng/μL of total RNA as starting material.



Purpose of Low Sample Input protocol

There are certain situations where the RNA yield is limited and low RNA input is desirable. Life Technologies has developed a Low Sample Input protocol for these types of samples with incorporation of a pre-amplification step prior to the final real-time PCR step.

Pre-amplification enables users to analyze a limited sample in more real-time PCR reactions without introducing any amplification bias. The pre-amplification step is performed using TaqMan® PreAmp Master Mix and pooled gene-specific primers.

Perform the reverse transcription

1. Thaw the SuperScript® VILO™ cDNA Synthesis Kit components and the RNA sample on ice.
2. Prepare the RT master mix by combining the following components for the number of reactions required.

Component	Volume ^[1] Pathways Panels (672-format)	Volume ^[1] Kinome Panel (896-format)
5X VILO™ Reaction Mix	4.0 µL	4.8 µL
10X SuperScript® Enzyme Mix	2.0 µL	2.4 µL
RNase-free water	4.0 µL	4.8 µL
RT master mix final volume	10.0 µL	12.0 µL

^[1] Add 10% overage to account for pipetting error.

3. Add 10 µL (for the Pathway Panels) or 12 µL (for the Kinome Panel) of sample RNA to the RT master mix.
4. Incubate at 25°C for 10 minutes.
5. Incubate at 42°C for 60 minutes.
6. Incubate at 85°C for 5 minutes, place on ice for 5 minutes, then spin down the contents of the microtube.

STOPPING POINT If needed, the obtained cDNA can be stored at -20°C for 2 months.

Perform the cDNA pre-amplification

TaqMan® PreAmp Primers pools for Pathway Panels are available as Pool A and Pool B, each sufficient for use with 100 samples.

1. Prepare 2 separate pre-amplification reactions for Pool A and for Pool B in 0.2 mL micro-centrifuge tubes or in 96-well plates by combining the following components.

Component	Pool A	Pool B
2X TaqMan® PreAmp Master Mix	10 µL	10 µL
TaqMan® PreAmp Pool	5 µL of Pool A	5 µL of Pool B
cDNA	5 µL	5 µL
Total	20 µL	20 µL

2. Cap the micro-centrifuge tubes or seal the 96-well plates with an adhesive film.
3. Mix the reactions by gently inverting the tube or swirling the plate, then centrifuge briefly.
4. Load the plate or tubes in a thermal cycler.
5. Run the following thermal cycler program:

	Stage	Cycles	Temperature	Time
Enzyme activation (hold)	1	1	95°C	10 minutes
PCR (cycle)	2	12-18 ^[1]	95°C	15 seconds
	3		60°C	4 minutes
Hold	4	1	99°C	10 minutes

^[1] Number of cycles depends on the starting RNA concentration (see Table 2).

6. Place the plates or the tubes on ice at the end of the run.

STOPPING POINT Pre-amplification products can be stored at –20°C for later use.

7. Mix Pool A and Pool B pre-amplification products of the same sample into one tube.
8. Dilute 1:20 the Pool A-Pool B mix.

Perform the PCR

1. Prepare the PCR master mix by combining the following components for 1 OpenArray® plate.

Component	Volume ^[1] 1 subarray	Volume ^[1] 12 subarrays	Volume ^[1] 16 subarrays
2X TaqMan® OpenArray® Real-Time Master Mix	2.5 µL	35 µL	45 µL
Mixed Pre-amplification product (diluted 1:20)	2.5 µL	35 µL	45 µL
Total	5 µL	70 µL	90 µL

^[1] 10% overage is already included in the volumes above to account for pipetting errors.

2. Mix the contents thoroughly by pipetting up and down 10 times.

3. Distribute 5 μ L of PCR reactions into each well of the 384-well sample plate using a single- or multi-channel pipette. The contents of one well of a 384-well sample will load one subarray on the OpenArray[®] plate (Figure 4 or Figure 5).
4. Cover the plate with sealing tape.
5. Centrifuge the sample plate for 1 minute at 1000 rpm to eliminate bubbles, then place the sample plate on ice.
6. Load the samples with the master mix from the 384-well sample plate onto the OpenArray[®] Plate using the OpenArray[®] AccuFill[™] System. For more information, consult *QuantStudio[™] 12K Flex Real-Time PCR System OpenArray[®] Experiments Getting Started Guide* (Pub. no. 4470935).
7. Run the PCR on the QuantStudio[™] 12K Flex Real-Time PCR System using the appropriate .edt or .tpf file containing all the information pertaining to the assays. For more information, consult *QuantStudio[™] 12K Flex Real-Time PCR System OpenArray[®] Experiments Getting Started Guide* (Pub. no. 4470935).



Gene-specific reverse transcriptions (optional)

Gene-specific reverse transcription reactions

For very low sample input (< 50 ng/μL), Life Technologies recommends performing a gene-specific reverse-transcription (RT) reaction before the pre-amplification step.

Component	VILO™ RT Master Mix Volume ^[1]	VILO™ RT Master Mix Volume for Kinome Panel ^[1]
5X VILO™ Enzyme Buffer	4 μL	4.8 μL
10X VILO™ Enzyme Mix	2 μL	2.4 μL
TaqMan® PreAmp Pool	2 μL (1 μL Pool A + 1 μL Pool B)	2.4 μL (1.2 μL Pool A + 1.2 μL Pool B)
Nuclease-free water	2 μL	2.4 μL
Total	10 μL	12 μL
Sample RNA	10 μL	12 μL
Reaction final volume	20 μL	24 μL

^[1] Add 10% overage to account for pipetting error.

Perform the gene-specific reverse transcription

1. Incubate at 25°C for 10 minutes.
2. Incubate at 42°C for 60 minutes.
3. Stop the reaction by incubating at 85°C for 5 minutes.
The cDNA is then pre-amplified using 2× TaqMan® PreAmp Master Mix and TaqMan® PreAmp Pools (“Perform the cDNA pre-amplification” on page 16).



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, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
-

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, and 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. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may 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/biosafety/publications/bmb15/BMBL.pdf
 - World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
-

Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

Note: For the SDSs of chemicals not distributed by Life Technologies Corporation, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (techsupport@lifetech.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

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