TaqMan[®] RNase P Detection Reagents Kit

100 reactions

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Applied Biosystems[™] TaqMan[®] RNase P Detection Reagents Kit contains a 20X mix of primers and probe used to detect and quantify genomic copies of the human RNase P gene. These reagents can be used with Applied Biosystems[™] Real-Time PCR systems.

IMPORTANT! The reagents are not compatible with StepOne[™] Real-Time PCR System.

The primers and probe are designed according to Primer Express[™] guidelines for quantification and utilize standard thermal cycling parameters. This kit is designed to perform a 5' nuclease assay with TaqMan[®] Universal PCR Master Mix with genomic DNA (gDNA), plasmid DNA, or complementary DNA (cDNA).

Contents and storage

Contents	Amount	Storage	
20X RNase P Primer-Probe (FAM [™] Dye) Mix	250 μL	0500 + 4500	
Human Genomic Control DNA, 10 ng/µL	100 µL		

Materials required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source	
Real-time PCR Instrument, one of the following:		
QuantStudio [™] 3 or 5 Real-Time PCR Instrument		
QuantStudio™ 6 / QuantStudio™ 7 Flex Instrument		
QuantStudio [™] 12K Flex Real-Time PCR System	Contact your local sales office.	
Step0nePlus [™] Real-Time PCR System		
7500/7500 Fast Real-Time PCR System		
Equipment		
Benchtop microcentrifuge	MLS	
Plate centrifuge	MLS	
Laboratory mixer (Vortex or equivalent)	MLS	



Item	Source	
Tubes, plates, and other consumables		
MicroAmp [™] Optical Adhesive Film, 100 covers	4311971	
Aerosol-resistant pipette tips		
96-well Standard plates (0.2 mL), 96-well Fast plates (0.1 mL), 384-well Standard plates and MicroAmp™ Reaction Tubes (0.1 mL and 0.2 mL)	thermofisher.com/plastics	
Reagents		
Nuclease-free Water	AM9938	
Master Mix, one of the following:		
TaqMan® Fast Advanced Master Mix	4444556	
TaqMan® Universal PCR Master Mix	4304437	
TaqMan® Universal Master Mix II	4440038	
TaqMan® Gene Expression Master Mix	4369016	

Methods

Prepare the PCR Reaction Mix

The detection range for Human Genomic Control DNA is between 50 ng to 50 pg per 20 µL reaction.

1. Prepare the PCR Reaction Mix in an appropriately sized microcentrifuge tube according to the following table.

Commenced	Volume per reaction ^[1]		
Component	384-well plate, 96-well Fast plate ^[2]	96-well Standard plate	
Master Mix (2X) ^[3]	5 µL	10 µL	
20X RNase P Primer-Probe (FAM [™] Dye) Mix	0.5 µL	1 µL	
cDNA Template ^[4] or Human Genomic Control DNA	1 µL	2 µL	
Nuclease-free Water	3.5 µL	7 μL	
Total PCR Reaction Mix volume	10 µL	20 µL	

^[1] Add 10% overage for pipetting loss.

^[2] When using TaqMan[®] Gene Expression Master Mix, TaqMan[®] Universal PCR Master Mix, TaqMan[®] Universal Master Mix II, use Standard mode thermal cycling conditions.

^[3] Recommended: TaqMan[®] Fast Advanced Master Mix.

^[4] The recommended amount of cDNA for this assay is 1-100ng. The recommended amount of gDNA is 10 ng for 10 µL reaction and 20 ng for 20 µL reaction.

2. Vortex the tube to mix the contents thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.

3. Add the PCR Reaction Mix to the appropriate wells of the reaction plate.

4. Seal the plate with optical adhesive film, then vortex briefly to mix.

5. Centrifuge the plate briefly to collect the contents at the bottom of the wells.

Set up the thermal protocol

See the appropriate instrument user guide for more detailed instructions to program the thermal cycling conditions or to run the plate.

1. Select the cycling mode appropriate for the Master Mix.

The cycling mode depends on the Master Mix that is used in the reaction and not on the Standard or Fast plate format.

2. Set up the thermal protocol for your instrument.

Table 2 TaqMan® Fast Advanced Master Mix (StepOnePlus[™] system, and QuantStudio[™] systems with fast cycling mode).

Step	Temperature	Time	Cycles
UNG Incubation	50°C	2 minutes	1
Enzyme Activation	95°C	2 minutes	1
Denature	95°C	1 second	(0
Anneal/Extend	60°C	20 seconds	40

Table 3 TaqMan® Fast Advanced Master Mix (7500 Fast and 7500 Real-Time PCR System with fast cycling mode).

Step	Temperature	Time	Cycles
UNG Incubation	50°C	2 minutes	1
Enzyme Activation	95°C	2 minutes	1
Denature	95°C	3 seconds	(0
Anneal/Extend	60°C	30 seconds	40

Table 4 TaqMan[®] Gene Expression Master Mix, TaqMan[®] Universal PCR Master Mix, or TaqMan[®] Universal Master Mix II (Any compatible instrument. Use standard cycling mode).

Step	Temperature	Time	Cycles
UNG Incubation	50°C	2 minutes	1
Enzyme Activation	95°C	10 minutes	1
Denature	95°C	15 seconds	(0
Anneal/Extend	60°C	60 seconds	40

3. Load the plate into the real-time PCR instrument, then start the run.

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 at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.



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

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Revision history: Pub. No. MAN0018429

Revision	Date	Description
B.0	12 March 2019	Corrected concentration of Human Genomic Control DNA.
А	8 January 2019	New Document. This document supersedes the <i>TaqMan®</i> <i>RNase P Detection Reagents (FAM[®] Dye) Product</i> <i>Information Sheet</i> (Pub. No. 4316834)

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