

RNase P Quantification for Genotyping Experiments

Calibrator method for quantification of purified DNA using a TaqMan™ RNase P assay

Pub. No. MAN0014349 Rev. B.0

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.

In this procedure, the quantification method uses TaqMan™ Control Genomic DNA (human) as a calibrator instead of a standard curve. The OpenArray Genotyping Calculation Sheet calculates the concentration of test DNA samples with this calibrator method.

Note: The OpenArray Genotyping Calculation Sheet assumes duplicate RNase P reactions in a 384-well plate layout.

Download the OpenArray Genotyping Calculation Sheet from thermofisher.com/oaqrc.

Required materials

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.

Catalog numbers that appear as links open the web pages for those products.

Item	Cat. No.
TaqMan™ Copy Number Reference Assay, human, RNase P (20X)	4403326
TaqMan™ Control Genomic DNA (human) (ready for use)	4312660
TaqPath™ ProAmp™ Master Mix	A30865

Before you begin

- Determine the total number of reactions required by including the following items:
 - Test DNA samples
 - Calibrator DNA sample
 - No template control (NTC) reactions (use nuclease-free water in place of DNA sample)

Note: We recommend a minimum of two reactions for each sample. The OpenArray Genotyping Calculation Sheet is set up for duplicate reactions.

- Generate the RNase P Sample TXT file from the OpenArray Genotyping Calculation Sheet.

Set up and run the PCR, then quantify the DNA

- Prepare a reaction mix for the required number of reactions plus 10% overage.

Component	Volume per reaction ^[1]
TaqPath™ ProAmp™ Master Mix	5.0 µL
TaqMan™ Copy Number Reference Assay, human, RNase P (20X)	0.5 µL
Nuclease-free Water	2.5 µL
Total	8 µL

^[1] See the OpenArray Genotyping Calculation Sheet to calculate volumes for multiple reactions.

- Transfer 8 µL of the reaction mix to the wells of a 384-well PCR reaction plate.
- Add 2 µL of sample DNA, TaqMan™ Control Genomic DNA (human), or nuclease-free water to the appropriate wells.
- Seal the plate with optical film.
- Set up the real-time instrument with the following thermal cycling conditions and settings:

Step	Temperature	Time	Cycles
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal/Extend	60°C	60 seconds	

- Experiment type: **Standard Curve**
- Mode: **Standard**
- Reporter: **VIC™**
- Quencher: **TAMRA™**
- Passive reference: **ROX**

- To assign the sample names, import the RNase P Sample TXT file generated from the OpenArray Genotyping Calculation Sheet.

- Load the plate, then start the run.

Calculate concentration of test DNA samples

This section provides instructions if the `OpenArray Genotyping Calculation Sheet` is used. This tool is recommended but is optional. Microsoft™ Excel™ can be used without the information that is populated in the tool.

1. In the instrument software, for example, the QuantStudio™ 12K Flex Software, after the run is complete, deselect all but the **Results** tab, select a file location, then export the results.
2. Remove duplicate sample IDs from the exported data.
 - a. Open the exported data file in Microsoft™ Excel™.
 - b. Copy–paste sample names and their corresponding C_t mean values into a new sheet.
 - c. Select both columns and use the **Remove Duplicates** function.
3. Calculate the concentration of test DNA samples.
 - a. Copy–paste the Sample Names and Mean C_t values into the `OpenArray Genotyping Calculation Sheet`.
 - b. Enter the dilution factor of the test DNA samples into the `OpenArray Genotyping Calculation Sheet`.



Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, California 94566 USA
For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0014349

Revision	Date	Description
B.0	11 April 2022	<ul style="list-style-type: none">• The instructions to dilute the test DNA 1:10 in nuclease-free water were removed.• The TaqMan™ Genotyping Master Mix was replaced with the TaqPath™ ProAmp™ Master Mix.• Thermal cycling conditions were added.• The passive reference dye selection was updated to ROX™ dye when setting up the PCR.• Information was added to indicate that the <code>OpenArray Genotyping Calculation Sheet</code> is optional.
A.0	23 October 2015	New document.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2022 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a trademark of Roche Molecular Systems, Inc., used under permission and license. Microsoft and Excel are trademarks of Microsoft Corporation.