

TaqMan[®] Copy Number Assays

Pub. No. 4397424 Rev. D

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *TaqMan[®] Copy Number Assays User Guide* (Pub. No. 4397425). 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 TaqMan[®] Copy Number Assays. For detailed instructions, supplemental procedures, and troubleshooting, see the *TaqMan[®] Copy Number Assays User Guide* (Pub. No. 4397424).

Guidelines for preparation of gDNA

Guidelines for isolation of high-quality gDNA

The target template for copy number assays is purified genomic DNA (gDNA). Wet gDNA or dried-down gDNA can be used.

Guidelines to quantify the gDNA

Quantification of gDNA is recommended. Use one of the following methods:

- The method described in *RNase P Quantification for Genotyping Experiments* (Pub. No. MAN0014349).
- UV absorbance (A_{260}/A_{280}) measurements. An A_{260}/A_{280} ratio of greater than 1.7 is recommended for human and mouse gDNA.

The RNase P method is preferred because it is more accurate than UV absorbance, and it assesses sample quality.

Perform PCR amplification

Determine the number of sample types and replicates

The following samples are recommended on each plate:

- **Samples or Unknowns**—gDNA samples in which the copy number of the target is unknown.
- **No-Template Controls (NTC)**—A sample that does not contain a DNA template. It shows the background fluorescence and allows for the detection of contamination.
- **Reference sample**—A DNA sample with a known copy number for the target of interest.

Four replicates of each gDNA sample are recommended for reliable copy number calls.

Dilute the 60X assays

Dilute 60X assays to 20X with TE, pH 8.0. Store the diluted samples at 2–8°C for immediate use or at –25°C to –15°C for long-term storage. Minimize exposure to light.

Prepare the PCR reactions

Copy number assays can be used with either wet or dried-down DNA. Drying down the gDNA in plates is the most convenient method for an experiment that requires multiple plates using the same gDNA or for multiple experiments that use the same gDNA.

Select the workflow to prepare PCR reactions based on whether wet gDNA or dried-down gDNA is used.

Prepare the PCR reactions with wet gDNA

Normalize the wet gDNA sample concentration

Dilute each sample to 5 ng/μL with nuclease-free water or 1X TE buffer, pH 8.

This is a 5X stock solution.

Store the diluted samples at 4°C for immediate use or at –25°C to –15°C for long-term storage.

Prepare the PCR Reaction Mix

Thaw the copy number assays and the copy number reference assays. Gently vortex to mix, then centrifuge briefly to bring contents to the bottom of the tube.

Note: The following Master Mixes are recommended for copy number assays:

- TaqPath™ ProAmp™ Master Mix
- TaqMan® Genotyping Master Mix

1. Mix the Master Mix thoroughly but gently.
2. In an appropriately-sized centrifuge tube, prepare PCR Reaction Mix according to the following table.

Component	Volume per well ^[1]	
	384-well (0.1-mL) or 96-well fast (0.1-mL) plate	96-well standard (0.2-mL) plate
Master Mix (2X)	5.5 µL	11.0 µL
TaqMan® Copy Number Assay (20X)	0.55 µL	1.1 µL
TaqMan® Copy Number Reference Assay (20X)	0.55 µL	1.1 µL
Nuclease-free Water	2.2 µL	4.4 µL
Total PCR Reaction Mix volume	8.8 µL	17.6 µL

^[1] Includes 10% overage.

3. Vortex the tube to mix the PCR Reaction Mix thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.
4. Add the PCR Reaction Mix to the appropriate wells of the plate.
 - 384-well (0.1-mL) or 96-well fast (0.1-mL) plate—8 µL per well
 - 96-well standard (0.2-mL) plate—16 µL per well
5. Gently vortex the gDNA samples, then add the gDNA to the appropriate wells of the plate.
 - 384-well (0.1-mL) or 96-well fast (0.1-mL) plate—2 µL of gDNA per well
 - 96-well standard (0.2-mL) plate—4 µL of gDNA per well
6. Seal the reaction plate with optical adhesive film, then vortex briefly to mix the contents.
7. Centrifuge the plate briefly to collect the contents at the bottom of the well.

Proceed to “Set up and run the real-time PCR instrument” on page 3.

Prepare the PCR reactions with dried-down gDNA

Dilute and dry the gDNA

The same amount of gDNA must be added to the reaction plate to dry. However, gDNA samples do not need to be diluted to the same concentration to dry.

1. Transfer the gDNA into each well of a plate.
 - 384-well (0.1-mL) plate or 96-well fast (0.1-mL) plate—10 ng per well
 - 96-well standard (0.2-mL) plate—20 ng per well
2. Allow the sample to dry at room temperature in an amplicon-free location.

Store the diluted samples at 4°C for immediate use or at -25°C to -15°C for long-term storage.

Prepare the PCR reactions

Thaw the copy number assays and the copy number reference assays. Gently vortex to mix, then centrifuge briefly to bring contents to the bottom of the tube.

Note: The following Master Mixes are recommended for copy number assays:

- TaqPath™ ProAmp™ Master Mix
- TaqMan® Genotyping Master Mix

1. Mix the Master Mix thoroughly but gently.
2. In an appropriately-sized centrifuge tube, prepare PCR Reaction Mix according to the following table.

Component	Volume per well ^[1]	
	384-well (0.1-mL) or 96-well fast (0.1-mL) plate	96-well standard (0.2-mL) plate
Master Mix (2X)	5.5 µL	11.0 µL
TaqMan® Copy Number Assay (20X)	0.55 µL	1.1 µL
TaqMan® Copy Number Reference Assay (20X)	0.55 µL	1.1 µL
Nuclease-free Water	4.4 µL	8.8 µL
Total PCR Reaction Mix volume	11.0 µL	22.0 µL

^[1] Includes 10% overage.

3. Vortex the tube to mix the PCR Reaction Mix thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.
4. Add the PCR Reaction Mix to each well of the reaction plate containing the dried-down gDNA.
 - 384-well (0.1-mL) or 96-well fast (0.1-mL) plate—10 µL per well
 - 96-well standard (0.2-mL) plate—20 µL per well
5. Seal the reaction plate with optical adhesive film, then vortex briefly to mix the contents.
6. Centrifuge the plate briefly to collect the contents at the bottom of the well.

Proceed to “Set up and run the real-time PCR instrument” on page 3.

Note: If the plate is not run immediately, protect it from light and store at 2–8°C. If necessary, the plate can be stored for up to 72 hours if using TaqPath™ ProAmp Master Mix.

Set up and run the real-time PCR instrument

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

Note: The instrument must be configured with the block appropriate for the plate type.

1. Set the experiment properties.
 - Block—**384-well** or **96-well fast (0.1 mL)** or **96-well standard (0.2 mL)**
 - Experiment type—**Standard curve**
 - Reagents—**TaqMan Reagents**
 - Properties—**Standard**

2. Define the targets and the samples, detectors, reporters and quenchers.

Table 1 Required instrument setup information

Instrument	Detector name	Target name	Reporter	Quencher
For copy number assays				
7500/7500 Fast Real-Time PCR Systems (software v2.X) QuantStudio™ 3 and 5 Real-Time PCR Systems QuantStudio™ 6 and 7 Flex Real-Time PCR Systems QuantStudio™ 12K Flex Real-Time PCR System ViiA™ 7 Real-Time PCR System StepOnePlus™ Real-Time PCR System	NA	User-defined	FAM™ dye	MGB-NFQ
7500/7500 Fast Real-Time PCR Systems (software v1.X) ^[1]	FAM™ dye	N/A	FAM™ dye	None
7900HT Fast Real-Time PCR Systems (software v2.X)	User-defined	N/A	FAM™ dye	Nonfluorescent
For copy number reference assays				
7500/7500 Fast Real-Time PCR Systems (software v2.X) QuantStudio™ 3 and 5 Real-Time PCR Systems QuantStudio™ 6 and 7 Flex Real-Time PCR Systems QuantStudio™ 12K Flex Real-Time PCR System ViiA™ 7 Real-Time PCR System StepOnePlus™ Real-Time PCR System	NA	User-defined	VIC™ dye	TAMRA™ probe
7500/7500 Fast Real-Time PCR Systems (software v1.X) ^[1]	VIC™ dye	NA	VIC™ dye	TAMRA™ probe
7900HT Fast Real-Time PCR Systems (software v2.X)	User-defined	NA	VIC™ dye	TAMRA™ probe

^[1] For SDS Software v1.X, specify FAM™ and VIC™ as the detector names for the copy number assay. SDS Software v1.X does not export dye information, so the reporter dyes must be specified in the detector name.

3. Assign the targets and samples to each well.

4. Set up the thermal protocol.

Note: PCR conditions are the same for TaqPath™ ProAmp™ Master Mix and TaqMan® Genotyping Master Mix. TaqPath™ ProAmp™ Master Mix contains heat-labile UNG, which is active during the reaction setup and is completely inactivated during the first ramp to the 95°C hold step.

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

5. Load the plate into the real-time PCR instrument.

6. Start the run.

Analyze and export results

Analyze the results

On the instrument, open the **Analysis Settings** window and use the following settings:

- Manual C_t threshold: **0.2**
- Autobaseline: **On**

Apply the settings, close the window, and run the experiment analysis.

For detailed information about data analysis, see the appropriate documentation for your instrument.

The general guidelines for copy-number data analysis include the following parameters:

- Ensure that the amplification curves for the copy number reference assay (VIC™ dye signal) in all samples have a distinct linear amplification phase.
- Ensure that the amplification curves for the copy number assay (FAM™ dye signal) in most wells have a distinct linear amplification phase.

Note: Samples that contain zero copies of the target of interest do not amplify well, if at all, with the copy number assay. Such samples have high or undetermined FAM™ C_t values.

- Review any displayed quality check (QC) flags, then review the real-time data of the associated samples.

Export the results

Export the results files for analysis with the CopyCaller™ Software. For detailed instructions, see *CopyCaller™ Software v2.0 User Guide* (Pub. No. 4400042).

Note: If using the ViiA™ 7 Software v1.0 and the CopyCaller™ Software v2.0 for copy number data analysis, export results using the 7900 Format option. The CopyCaller™ Software cannot open ViiA™ 7 Software v1.0 export files in other formats.

IMPORTANT! Do not modify the exported data files. The CopyCaller™ Software might not be able to import files that have been modified.

Guidelines to export the results

IMPORTANT! If you run multiple copy number assays or copy number reference assays on each plate, apply distinct assay detector and target names to the document. See details in “Set up and run the real-time PCR instrument” on page 3. User-defined target and detector names allow the CopyCaller™ Software to arrange and analyze data independently for each assay in a single exported file.

IMPORTANT! If you use a 7500/7500 Fast Real-Time PCR System running SDS Software v1.X, you must specify FAM™ dye and VIC™ dye as the detector names for the copy number assays and copy number reference assays, respectively. SDS Software v1.X does not export dye information, so you must specify the reporter dye in the detector name.

Assays per plate	Assay placement specified with separate target and detector	Procedure
1	—	Export the real-time PCR results to a tab-delimited text (TXT) file or comma-separated values (CSV) file.
>1	Yes	Export the real-time PCR results to a single exported file (CSV or TXT) that includes all wells of the plate. The CopyCaller™ Software uses the target and detector names to distinguish the data from the different assays.
	No	Export the real-time PCR results of each assay to a separate exported file. <ol style="list-style-type: none">1. Select the wells of the plate that contain the data from one of the copy number assays.2. Select File ▶ Export, then export the data from the selected wells to a data file.3. Repeat to export the data from the other assays present on the plate. Note: To help with organization, name each exported file according to the assay data it contains.

Import the results to the CopyCaller™ Software

For more information, see the *CopyCaller™ Software v2.0 User Guide* (Pub. No. 4400042).

1. Import the exported real-time PCR results file into the software.
2. Run the analysis to determine the copy number for your target in each sample.



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Products:
 TaqMan Copy Number Assays
 Custom TaqMan Copy Number Assays
 Custom Plus TaqMan Copy Number Assays
 TaqMan Copy Number Reference Assay, human, RNase P
 TaqMan Copy Number Reference Assay, human, TERT
 TaqMan Copy Number Reference Assay, mouse, Tfrc
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Products:
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Revision	Date	Description
D	6 February 2019	<ul style="list-style-type: none"> Added new instruments, Master Mixes, and other applicable products. Added details for dilution of 60X assays. Added storage information for gDNA. Updated for general style, formatting, and branding. Removed ordering information.
C	September 2010	Baseline for this revision history

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