TaqMan® Copy Number Assays

For safety and biohazard guidelines, refer to the "Safety" section in the *TaqMan® Copy Number Assays Protocol* (PN 4397425). For all chemicals, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare the DNA and Design the Experiment

1 Extract the DNA

The target template for TaqMan® Copy Number Assays is purified genomic DNA (gDNA).

Applied Biosystems recommends using commercially available gDNA extraction and purification kits.

2 Quantify the DNA

Applied Biosystems strongly recommends that you quantify the gDNA using one of the following methods:

- The TaqMan® RNase P Detection Reagents (PN 4316831) for human gDNAs. You can use your own human DNA samples or the TaqMan® DNA Template Reagents (PN 401970) to create a standard curve.

  or

- UV absorbance (A_{260}/A_{280}) measurements. Ensure that the human or mouse gDNA that you use has an A_{260}/A_{280} ratio greater than 1.7.

Note: The TaqMan® Copy Number Reference Assay RNase P method is preferred because it is more accurate than UV absorbance, and it assesses sample quality.

3 Dilute the DNA and prepare the plates

Follow the instructions for the type of gDNA you are using:

- **For liquid gDNA** – Dilute each sample to 5 ng/µL using either nuclease-free water or 1× TE buffer, pH 8.0 to make a 5× stock solution.
- **For dried gDNA** – You do not need to dilute all the DNA samples to the same working stock concentration. However, you must load the same amount of gDNA in each well of the plate. See the example in the chart below.

For a...	Your final reaction volume is...	The amount of gDNA required per well is...	The volume of 5 ng/µL (5×) gDNA stock per well is...
384-well plate	10 µL	10 ng	2 µL
96-well plate	20 µL	20 ng	4 µL

IMPORTANT! You must use the same amount of gDNA for each sample and for each sample replicate that is run with the same assay.

4 Determine the number of sample types for the experiment

Applied Biosystems recommends running the following samples 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.
- **Calibrator sample** – A DNA sample with a known copy number for the target of interest. Also known as the reference sample.

IMPORTANT! To generate the reliable copy number calls, Applied Biosystems strongly recommends using *four replicates* for each gDNA sample, on the plate, in order.

Prepare the Reactions for Liquid gDNA

1 Calculate the volume of the reaction components

Calculate the volumes of components that you need, based on the reaction volume and the number of reactions. Include excess volume in your calculations to provide for the loss that occurs during reagent transfers.

Note: Applied Biosystems recommends using *four replicates* of each sample.

Reaction mixture component	Volume per well (µL)	
	384-well plate	96-well plate
2× TaqMan® Genotyping Master Mix‡	5.0	10.0
TaqMan® Copy Number Assay, 20× working stock§	0.5	1.0
TaqMan® Copy Number Reference Assay, 20×	0.5	1.0
Nuclease-free water	2.0	4.0
Total Volume	**8.0**	**16.0**

‡ TaqMan® Gene Expression or TaqMan® Universal Master Mixes can also be used, but *do not* use TaqMan® Fast Universal Master Mix.
§ If you use large-scale assays (60×), dilute the assays to a 20× working stock.

2 Thaw the assays and combine with Master Mix

a. Completely thaw the TaqMan Copy Number Assays and the TaqMan® Copy Number Reference Assays. Gently vortex the assays to mix them, then centrifuge the tubes briefly to bring contents to the bottom of the tube.

b. Swirl to thoroughly mix the TaqMan® Genotyping Master Mix.

c. Combine the required volumes of reaction components in microcentrifuge tubes.

d. Invert or flick the tubes to mix the contents thoroughly, then centrifuge the tubes briefly.

3 Add the gDNA and reaction mixture to the plate

a. Pipette the reaction mixture into the wells of the reaction plate that you prepared.
- For 384-well plates – pipette 8 µL per well.
- For 96-well plates – pipette 16 µL per well.

b. Vortex the gDNA samples that you prepared and diluted.

c. Add the gDNA to the wells containing the reaction mixture:
- For 384-well plates – pipette 2 µL of gDNA (5 ng/µL) per well.
- For 96-well plates – pipette 4 µL of gDNA (5 ng/µL) per well.

Alternatively, you can add the gDNA to the plate first, then add the reaction mixture.

4 Mix and seal the plate

a. Mix the reaction mixture with the gDNA by pipetting up and down several times.

b. Seal the reaction plate with optical adhesive film (or optical caps), then centrifuge the reaction plate briefly.

c. Inspect all the wells to ensure a uniform volume.

d. Follow the instructions in "Run the Plate and Analyze the Results" on page 4.

Prepare the Reactions for Dried gDNA

1 Dry down the gDNA

a. Transfer the gDNA into each well of a MicroAmp® Optical Reaction Plate:
   - For a 384-well plate – pipette 10 ng
   - For a 96-well plate – pipette 20 ng

b. Allow the sample to dry at room temperature in an amplicon-free location.

2 Calculate the volume of the reaction components

Calculate the volumes of components that you need, based on the reaction volume and the number of reactions. Include excess volume in your calculations to provide for the loss that occurs during reagent transfers.

Note: Applied Biosystems recommends using *four replicates* of each sample.

Reaction mixture component	Volume per well (µL)	
	384-well plate	96-well plate
2× TaqMan® Genotyping Master Mix‡	5.0	10.0
TaqMan® Copy Number Assay, 20× working stock§	0.5	1.0
TaqMan® Copy Number Reference Assay, 20×	0.5	1.0
Nuclease-free water	4.0	8.0
Total Volume	**10.0**	**20.0**

‡ TaqMan® Gene Expression or TaqMan® Universal Master Mixes can also be used, but *do not* use TaqMan® Fast Universal Master Mix.
§ If you use large-scale assays (60×), dilute the assays to a 20× working stock.

3 Thaw the assays and combine with Master Mix

a. Completely thaw the TaqMan Copy Number Assays and the TaqMan® Copy Number Reference Assays. Gently vortex the assays to mix them, then centrifuge the tubes briefly to bring contents to the bottom of the tube.

b. Swirl to thoroughly mix the TaqMan® Genotyping Master Mix.

c. Combine the required volumes of reaction components in microcentrifuge tubes.

d. Invert or flick the tubes to mix the contents thoroughly, then centrifuge the tubes briefly.

4 Add the reaction mixture to the dried gDNA

a. Obtain the reaction plate containing the dried gDNA.

b. Pipette the reaction mixture into each of the wells of the reaction plate.
   - For 384-well plates – pipette 10 µL per well.
   - For 96-well plates – pipette 20 µL per well.

5 Mix and seal the plate

a. Mix the reaction mixture with the gDNA by pipetting up and down several times.

b. Seal the reaction plate with optical adhesive film (or optical caps), then centrifuge the reaction plate briefly.

c. Inspect all the wells to ensure a uniform volume.

d. Follow the instructions in "Run the Plate and Analyze the Results" on page 4.

Run the Plate and Analyze the Results

1 Create the plate document/experiment

a. Create a plate document/experiment for the run using the parameters in the following table:

System	Run	Reaction plate	Ramp speed/ model	Sample volume
ViiA™ 7 Real-Time PCR System (ViiA™ 7 Software v1.X; 7500 Software v2.0 or later)	Standard	96-well standard	Standard	20 µL
		96-well Fast		
		384-well standard		10 µL
7900HT/7900HT Fast (SDS Software v2.1 or later)	Standard	96-well standard	9600 emulation	20 µL
		96-well Fast (for 7900HT)		
		384-well standard (for 7900HT)		10 µL
7500/7500 Fast (SDS Software v1.3 or later)	Standard	96-well standard	9600 emulation	20 µL
		96-well Fast		
7500/7500 Fast (7500 Software v2.0 or later)	Standard	96-well standard	Standard	20 µL
		96-well Fast		
7300 (SDS Software v1.3 or later)	Standard	96-well standard	9600 emulation	20 µL
StepOnePlus™ (StepOne™ Software v2.0 or later)	Standard	96-well Fast	Standard	20 µL

b. In the plate document, identify the samples in each well of the reaction plate:

- Select the wells containing gDNA and the no template controls (NTCs).
- Apply to each well of the plate that contains a reaction: a sample name and a detector/target that includes dye information (reporter and quencher).
- Create unique assay sample names so that the CopyCaller™ Software analyzes each sample separately.
- Apply the same sample name to the wells of each technical replicate group.
- Apply unique detector/target names to the wells of plates that contain multiple TaqMan Copy Number Assays or Reference Assays (optional).
- Apply the setup data shown in the table below for your Real-Time PCR System to each TaqMan® Copy Number experiment.

IMPORTANT! The shaded cells in the table below indicate that you must enter the specified values exactly as shown.

1 Create the plate document/experiment (continued)

Required setup information for a TaqMan® Copy Number Assay

Assay	Detector name	Target name	Reporter	Quencher
7900HT Fast System (SDS Software v2.X) – Absolute quantitation plate document‡				
TaqMan® Copy Number Assay	User-defined	N/A	FAM	Nonfluorescent
TaqMan® Copy Number Reference Assay	User-defined	N/A	VIC	TAMRA
7300/7500/7500 Fast System (SDS Software v1.X) – Absolute quantitation plate document				
TaqMan® Copy Number Assay	FAM§	N/A	FAM	(none)
TaqMan® Copy Number Reference Assay	VIC§	N/A	VIC	TAMRA
7500/7500 Fast System (7500 Software v2.X) or **ViiA™ 7 Software v1.X** or **StepOnePlus™ System (StepOne Software v2.*X*) – Advanced Setup/Quantitation-Standard Curve experiment‡**				
TaqMan® Copy Number Assay	N/A	User-defined	FAM	NFQ-MGB
TaqMan® Copy Number Reference Assay	N/A	User-defined	VIC	TAMRA

‡ If you run more than one TaqMan® Copy Number or Reference Assay on a plate, you can enter the names of the assays in the Detector/Target Name fields so that the CopyCaller™ Software analyzes the data from each assay separately.

§ If you use SDS Software v1.*X*, you must specify "FAM" and "VIC" as the detector names for the Copy Number and Reference Assays respectively. SDS Software v1.*X* does not export dye information, so you must specify the reporter dye(s) in the detector name.

2 Run the plate

a. Load the reaction plate into a real-time PCR instrument.

b. Run the plate using the parameters below:

Stage	Temperature	Time
Hold	95 °C	10 min
Cycle (40 Cycles)	95 °C	15 sec
	60 °C	60 sec

c. Unload the reaction plate after the run is complete.

3 Analyze the results

a. In the real-time PCR Instrument software, open the Analysis Settings window and set the following:
- Manual C_T threshold – **0.2**
- Autobaseline – **On**

b. Apply the settings, then close the window.

c. Analyze the experiment.

d. Review the analyzed data and troubleshoot any flags or problematic data. Verify that the amplification curves for the:
- Reference Assay (VIC® dye signal) in all samples have a distinct, linear amplification phase.
- 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_Ts.

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

4 Export the results: In the Real-Time PCR Instrument software

Assays per plate	Did you specify the placement of the assay(s) using separate targets/ detectors?	Procedure
One	Not applicable	Export the real-time PCR results to a tab-delimited text (.txt) or comma-separated values (.csv) exported file.
More than one	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/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. 1. Select the wells of the plate that contain the data from one of the TaqMan® Copy Number Assays. 2. Select **File ▸ Export**, then export the data from the selected wells to a data file. 3. Repeat steps 1 and 2 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.

5 Export the results: In the CopyCaller™ Software

a. Import the exported real-time PCR file into the CopyCaller™ Software.

b. Run the analysis to determine the copy number for your target in each sample.

Ordering Information

TaqMan® Copy Number Assays, Custom Plus TaqMan® Copy Number Assays and Custom TaqMan® Copy Number Assays

Scale	Concentration	Number of reactions		Part number		
		384-well, 10 µL	96-well, 20 µL	Pre-designed assays	Custom Plus assays	Custom assays
Small	20×	720	360	4400291	4442487	4400294
Medium	20×	1500	750	4400292	4442520	4400295
Large	60×	5800	2900	4400293	4442488	4400296

TaqMan® Copy Number Reference Assays

Product	Concentration	Number of reactions		Part number
		384-well, 10 µL	96-well, 20 µL	
Human Assays				
TaqMan® Copy Number Reference Assay RNase P, 750 Reactions	1 tube, 20×	1500	750	4403326
TaqMan® Copy Number Reference Assay RNase P, 3000 Reactions	4 tubes, 20×	6000	3000	4403328
TaqMan® Copy Number Reference Assay TERT, 750 Reactions	1 tube, 20×	1500	750	4403316
TaqMan® Copy Number Reference Assay TERT, 3000 Reactions	4 tubes, 20×	6000	3000	4403315
Mouse Assays				
TaqMan® Copy Number Reference Assay, Mouse, Tfrc, 750 Reactions	1 tube, 20X	1500	750	4458366
TaqMan® Copy Number Reference Assay, Mouse, Tfrc, 3000 Reactions	4 tubes, 20X	6000	3000	4458367
TaqMan® Copy Number Reference Assay, Mouse, Tert, 750 Reactions	1 tube, 20X	1500	750	4458368
TaqMan® Copy Number Reference Assay, Mouse, Tert, 3000 Reactions	4 tubes, 20X	6000	3000	4458369

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Part Number 4397424 Rev. C 09/2010

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