

TaqMan™ Copy Number Assays

USER GUIDE

Publication Number 4397425

Revision F

**Manufacturer:**

Life Technologies Holdings Pte Ltd |
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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
 TaqMan Copy Number Reference Assay, mouse, TERT

**Manufacturer:**

Life Technologies Corporation |
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 Pleasanton, CA 94566

Products:

TaqMan Copy Number Assays
 Custom TaqMan Copy Number Assays
 Custom Plus TaqMan Copy Number Assays

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| Revision | Date | Description |
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| F | 10 December 2019 | Removed troubleshooting and replaced with link to FAQs. |
| E | 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 chemistry information. Updated order and design information. Updated for general style, formatting, and branding. |
| D | September 2010 | Baseline for this revision history. |

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Product information

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Product description

Applied Biosystems™ TaqMan™ Copy Number Assays are designed to detect and measure copy number variation within the human and mouse genomes. Copy number variation is an important polymorphism associated with genetic diseases such as cancer, immune diseases, and neurological disorders.

- TaqMan™ Copy Number Assays
 - Include an extensive collection of pre-designed assays.
 - Are available for genes and known copy number variation regions.
 - Are available for human and mouse genomes.
 - Provide common vector marker and reporter genes for study of transfected cells and organisms.
- Custom Plus TaqMan™ Copy Number Assays
 - Include bioinformatics analysis on the target and assay sequences.
 - Can be designed for other targets of interest in human and mouse genomes.
- Custom TaqMan™ Copy Number Assays
 - Can be designed for other targets of interest.
 - Do not include bioinformatic analysis.
- TaqMan™ Copy Number Reference Assays
 - Are designed to unique human and mouse genomic sequences.
 - Are required for relative quantitation of copy number targets.

This document provides instructions for performing and analyzing copy number variation quantification experiments using the copy number assays and the copy number reference assays.

Assay description

The copy number assays are run simultaneously with a copy number reference assay in a duplex real-time polymerase chain reaction (PCR). The copy number assay detects the target gene or genomic sequence of interest, and the copy number reference assay detects a sequence that is known to exist in two copies in a diploid genome, for example, the human RNase P H1 RNA gene, RPPH1. This method of relative quantitation is used to determine the relative copy number of the target of interest in a genomic DNA (gDNA) sample, normalized to the known copy number of the reference sequence.

The number of copies of the target sequence in each test sample is determined by relative quantitation (RQ) using the comparative C_t ($\Delta\Delta C_t$) method. This method measures the C_t difference (ΔC_t) between target and reference sequences, then compares the ΔC_t values of test samples to a calibrator sample known to have two copies of the target sequence. The copy number of the target is calculated to be two times the relative quantity.

In a copy number quantification reaction, purified gDNA is combined with the copy number assay and the copy number reference assay.

The copy number assay includes the following components:

- Two unlabeled primers for amplifying the target sequence of interest.
- One TaqMan™ MGB probe for detecting the target sequence of interest. The probe incorporates the following items:
 - A FAM™ reporter dye, attached to the 5' end.
 - A nonfluorescent quencher (NFQ) and a Minor Groove Binder (MGB), attached to the 3' end.

Note: MGBs increase the melting temperature (T_m) without increasing probe length. They allow for the design of shorter probes.

The copy number reference assay includes the following components:

- Two unlabeled primers for amplifying the reference sequence.
- One TaqMan™ TAMRA™ probe for detecting the reference sequence. The probe incorporates the following items:
 - A VIC™ reporter dye, attached to the 5' end.
 - A TAMRA™ quencher, attached to the 3' end.

Table 1 Description of the reference assays

| Assay | Purpose | Function |
|---|--|--|
| Human | | |
| TaqMan™ Copy Number Reference Assay, human, RNase P | Standard reference assay for human gDNA copy number quantitation experiments | Detects the Ribonuclease P RNA component H1 (H1RNA) gene (RPPH1) on chromosome 14, cytoband 14q11.2. |
| TaqMan™ Copy Number Reference Assay, human, TERT | Alternative reference assay for human gDNAs | Targets the telomerase reverse transcriptase (TERT) gene located on chromosome 5, cytoband 5p15.33. |

| Assay | Purpose | Function |
|--|--|--|
| Mouse | | |
| TaqMan™ Copy Number Reference Assay, mouse, Tfrc | Standard reference assay for mouse gDNA copy number quantitation experiments | Detects the transferrin receptor gene (Tfrc) on chromosome 16, cytoband 16qB3. |
| TaqMan™ Copy Number Reference Assay, mouse, TERT | Alternative reference assay for mouse gDNA | Targets the telomerase reverse transcriptase (TERT) gene located on chromosome 13, cytoband 13qC1. |

Contents and storage

TaqMan™ Copy Number Assays

Each assay contains target-specific forward and reverse primers and FAM™ dye-labeled MGB probe.

All of the assays are available in 20X and 60X concentrations and are made to order.

Table 2 TaqMan™ Copy Number Assays

| Size | Number of reactions | | Cat. No. | | | Shipping and storage |
|--------------|---------------------|----------------|----------------------------|-----------------------------------|--|---|
| | 10-μL reaction | 20-μL reaction | TaqMan™ Copy Number Assays | Custom TaqMan™ Copy Number Assays | Custom Plus TaqMan™ Copy Number Assays | |
| Small (20X) | 720 | 360 | 4400291 | 4400294 | 4442487 | Shipped at ambient temperature. Store at -25°C to -15°C. Minimize freeze-thaw cycles. |
| Medium (20X) | 1500 | 750 | 4400292 | 4400295 | 4442520 | |
| Large (60X) | 5800 | 2900 | 4400293 | 4400296 | 4442488 | |

Reference assay description

Each reference assay detects a single-copy gene in its respective reference genome assembly. Each reference assay contains reference sequence-specific forward and reverse primers and a VIC™ dye-labeled TAMRA™ probe.

The reference assays are ordered separately from the copy number assays. For a description of the reference assays, see Table 1 on page 6.

Table 3 Reference assays

| Product | Number of reactions | | Cat. No. | Shipping and storage |
|--|---------------------|-----------------|----------|---|
| | 10-μL reactions | 20-μL reactions | | |
| Human | | | | |
| TaqMan™ Copy Number Reference Assay, human, RNase P, 1 tube (20X) | 1500 | 750 | 4403326 | Shipped at ambient temperature. Store at -25°C to -15°C. Minimize freeze-thaw cycles. |
| TaqMan™ Copy Number Reference Assay, human, RNase P, 4 tubes (20X) | 6000 | 3000 | 4403328 | |
| TaqMan™ Copy Number Reference Assay, human, TERT, 1 tube (20X) | 1500 | 750 | 4403316 | |
| TaqMan™ Copy Number Reference Assay, human, TERT, 4 tubes (20X) | 6000 | 3000 | 4403315 | |

| Product | Number of reactions | | Cat. No. | Shipping and storage |
|---|-----------------------|-----------------------|----------|---|
| | 10- μ L reactions | 20- μ L reactions | | |
| Mouse | | | | |
| TaqMan™ Copy Number Reference Assay, mouse, Tfrc, 1 tube (20X) | 1500 | 750 | 4458366 | Shipped at ambient temperature. Store at -25°C to -15°C. Minimize freeze-thaw cycles. |
| TaqMan™ Copy Number Reference Assay, mouse, Tfrc, 4 tubes (20X) | 6000 | 3000 | 4458367 | |
| TaqMan™ Copy Number Reference Assay, mouse, TERT, 1 tube (20X) | 1500 | 750 | 4458368 | |
| TaqMan™ Copy Number Reference Assay, mouse, TERT, 4 tubes (20X) | 6000 | 3000 | 4458369 | |

Order copy number assays and reference assays

- Order TaqMan™ Copy Number Assays.
 - a. Go to [thermofisher.com/taqmancnv](https://www.thermofisher.com/taqmancnv).
 - b. Use the **Assay Search Tool**.
- Design and order Custom TaqMan™ Copy Number Assays and Custom Plus TaqMan™ Copy Number Assays, and order TaqMan™ Copy Number Reference Assays.
 - a. Go to [thermofisher.com/taqmancustomcnv](https://www.thermofisher.com/taqmancustomcnv).
 - b. Read the background material, then click **Order Custom TaqMan™ Copy Number Assays**.
 - c. Follow the prompts to search for or enter the sequences you need.

Master Mixes

The following Master Mixes are recommended for copy number assays:

- TaqPath™ ProAmp™ Master Mix for samples with PCR inhibitors such as heparin and hematin
- TaqMan™ Genotyping Master Mix

Table 4 TaqPath™ ProAmp™ Master Mix

| Cat. No. | Amount | Storage |
|----------|-----------|---------|
| A30865 | 1 x 1 mL | 2–8°C |
| A30866 | 1 x 10 mL | |
| A30871 | 2 x 10 mL | |
| A30867 | 1 x 50 mL | |
| A30872 | 2 x 50 mL | |

Table 5 TaqMan™ Genotyping Master Mix

| Cat. No. | Amount | Storage |
|----------|-----------|---------|
| 4371353 | 1 x 1 mL | 2–8°C |
| 4371355 | 1 x 10 mL | |
| 4381656 | 2 x 10 mL | |
| 4371357 | 1 x 50 mL | |
| 4381657 | 2 x 50 mL | |

The following Master Mixes are also compatible with copy number assays:

- TaqMan™ Gene Expression Master Mix
- TaqMan™ Universal PCR Master Mix
- TaqMan™ Universal PCR Master Mix, no AmpErase™ UNG
- TaqMan™ Universal Master Mix II

For details on PCR conditions for the Master Mixes see the appropriate user guide.

Note: TaqMan™ Fast Advanced Master Mix is not recommended for use with copy number assays.

Required materials not supplied

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

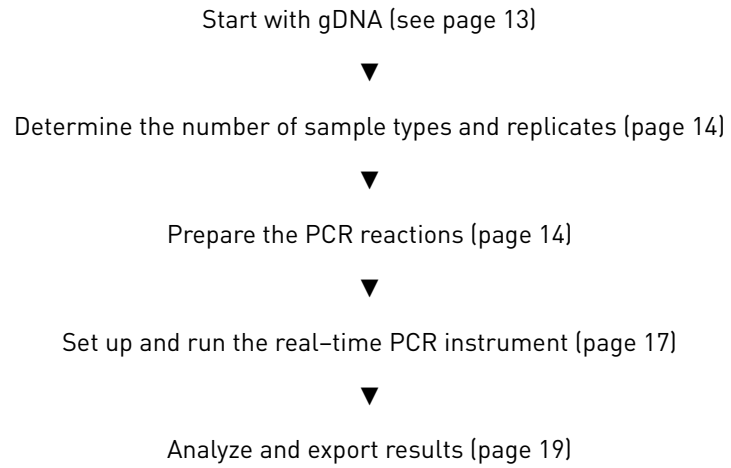
Table 6 Recommended products to prepare gDNA

| Item | Source |
|----------------------|--|
| Kits to prepare gDNA | thermofisher.com/gdnaprep |

Table 7 Other materials and equipment required for the workflow

| Item | Source |
|--|---|
| Real-time PCR instrument, one of the following: | |
| QuantStudio™ 3 or 5 Real-Time PCR System | Contact your local sales office |
| QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System | |
| QuantStudio™ 12K Flex Real-Time PCR System | |
| StepOnePlus™ Real-Time PCR System | |
| ViiA™ 7 Real-Time PCR System | |
| 7500/7500 Fast Real-Time PCR System | |
| 7900HT Fast Real-Time PCR System | |
| Software | |
| CopyCaller™ Software (recommended to analyze copy number assays) | https://www.thermofisher.com/us/en/home/technical-resources/software-downloads/copycaller-software.html |
| Equipment | |
| Centrifuge, with adapter for 96-well plates or 384-well plates | MLS |
| Microcentrifuge | MLS |
| Vortex mixer | MLS |
| Pipettes | MLS |
| Tubes, plates, and other consumables | |
| Tubes, plates, and film | thermofisher.com/plastics |
| Aerosol-resistant barrier pipette tips | MLS |
| Disposable gloves | MLS |
| Reagents | |
| Nuclease-free Water | AM9930 |
| TaqMan™ DNA Template Reagents | 401970 |
| TaqMan™ RNase P Detection Reagents Kit | 4316831 |
| (Optional) TE, pH 8.0 | AM9849 |

Workflow





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. For recommended DNA isolation kits see Table 6 on page 10.

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.

3

Perform PCR amplification

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- Dilute the 60X assays 14
- Prepare the PCR reactions 14
- Set up and run the real-time PCR instrument 17

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 1× TE buffer, pH 8. This is a 5× 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.

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 17.

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.

- 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
- 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.

- Mix the Master Mix thoroughly but gently.
- 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.

- Vortex the tube to mix the PCR Reaction Mix thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.
- 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
- Seal the reaction plate with optical adhesive film, then vortex briefly to mix the contents.
- 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 17.

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 8 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

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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 17. 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. <p>Note: To help with organization, name each exported file according to the assay data it contains.</p> |

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.



Troubleshooting and FAQs

Visit our online FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

- For troubleshooting information and FAQs for this product: [thermofisher.com/cnafaqs](https://www.thermofisher.com/cnafaqs)
- To browse the database and search using keywords: [thermofisher.com/faqs](https://www.thermofisher.com/faqs)



Supplemental information

TaqMan™ chemistry

About the 5' nuclease assay













A copy number assay, a copy number reference assay, a Master Mix, and a gDNA sample are combined in a single well or tube.

The gDNA template is denatured and each set of assay primers anneals to its specific target sequences. Each TaqMan™ probe anneals specifically to its complementary sequence between forward and reverse primer binding sites.

When each oligonucleotide probe is intact, the proximity of the quencher dye to the reporter dye causes the reporter dye signal to be quenched.

During each round of PCR, the target and reference sequences are simultaneously amplified by the DNA polymerase. This enzyme has a 5' nuclease activity that cleaves probes that are hybridized to each amplicon sequence.

When an oligonucleotide probe is cleaved by the DNA polymerase 5' nuclease activity, the quencher is separated from the reporter dye, increasing the fluorescence of the reporter. Accumulation of PCR products can be detected in real-time by monitoring the increase in fluorescence of each reporter dye at each PCR cycle.

- | | |
|---|--|
|  Forward primer |  FAM™ dye |
|  Reverse primer |  Fluorescing FAM™ dye |
|  gDNA |  VIC™ dye |
|  Probe |  Fluorescing VIC™ dye |
|  Minor groove binder probe |  TAMRA™ quencher |
|  Non-fluorescent quencher |  DNA polymerase |

The copy number assay is shown in Figure 1 through Figure 4.

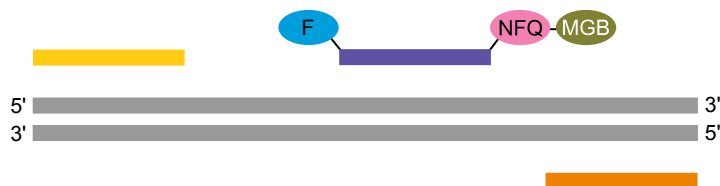


Figure 1 Copy number assay components

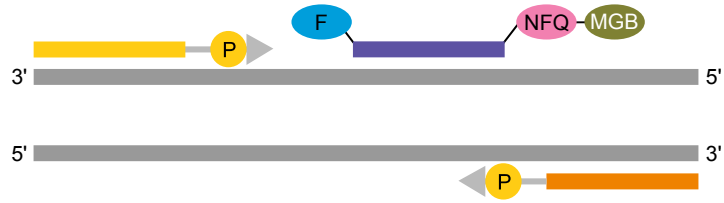


Figure 2 Denatured template and annealing of assay components

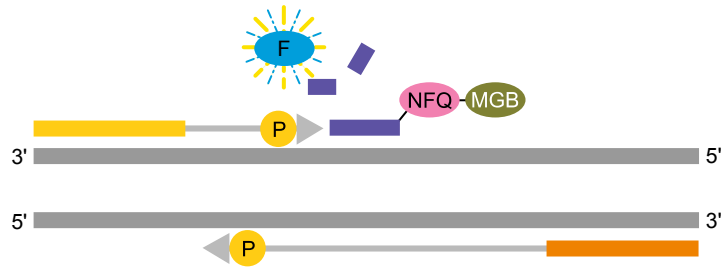


Figure 3 Polymerization of assay components

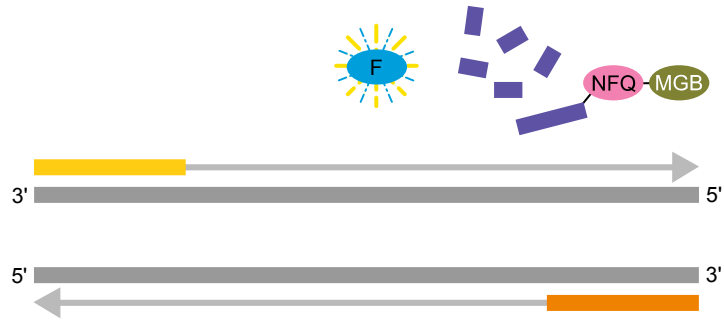


Figure 4 Completion of assay

The copy number reference assay is shown in Figure 5 through Figure 8.

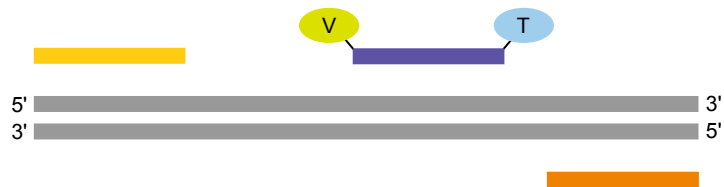


Figure 5 Copy number reference components

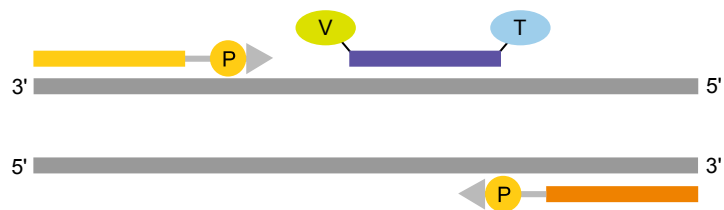


Figure 6 Denatured template and annealing of reference components

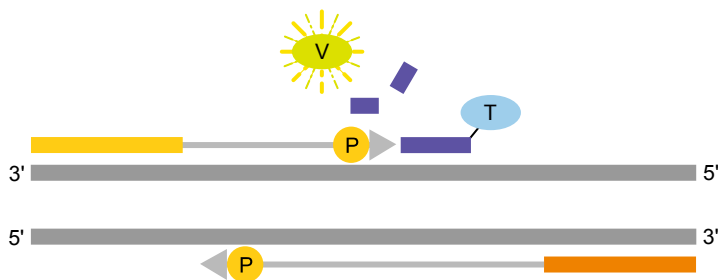


Figure 7 Polymerization of reference components

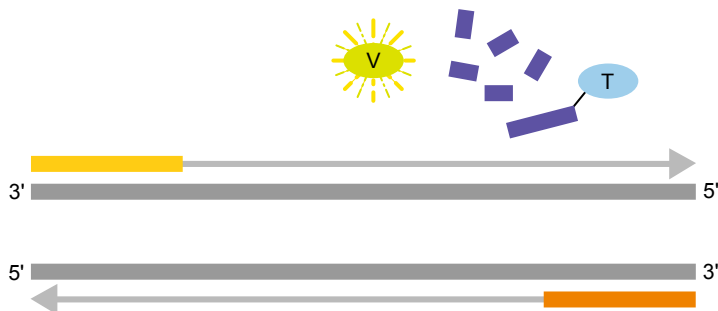


Figure 8 Completion of assay

Best practices for PCR and RT-PCR experiments

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
 - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
 - Sample preparation and reaction setup.
 - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Use UNG to prevent false-positive amplification

Carryover amplicons can result in false-positive amplification during PCR. Use a master mix that contains heat-labile uracil-N-glycosylase (UNG; also known as uracil-DNA glycosylase (UDG)) to degrade many contaminating carryover amplicons.

UNG enzymatic activity occurs during the PCR reaction setup at room temperature; an activation step before thermal cycling is not necessary. Unlike standard UNG, heat-labile UNG is completely inactivated during the first ramp to the high-temperature step for template denaturation and polymerase activation.

To ensure the desired UNG activity:

- Use PCR components and thermal cycling conditions as specified. UNG-containing master mixes incorporate the optimal concentration of UNG to prevent cross-contamination while not affecting real-time PCR performance.
- Do not attempt to use UNG-containing master mixes in subsequent amplification of dU-containing PCR products, such as in nested-PCR protocols. The UNG will degrade the dU-containing PCR products, preventing further amplification.

Although treatment with UNG can degrade or eliminate large numbers of carryover PCR products, use good laboratory practices to minimize cross-contamination from non-dU-containing PCR products or other samples.

Detect fluorescent contaminants

Fluorescent contaminants can generate false positive results. To help detect these contaminants, we recommend including a no-amplification control reaction that contains sample, but no master mix.

After PCR, if the absolute fluorescence of the no-amplification control is greater than the fluorescence of the no template control (NTC), fluorescent contaminants may be present in the sample or in the heat block of the real-time PCR instrument.



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, and so on). 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. 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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.



Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also 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:
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.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

Related documentation

| Document | Pub. No. |
|---|------------|
| <i>TaqMan™ Copy Number Assays Quick Reference</i> | 4397424 |
| <i>CopyCaller™ Software v2.0 User Guide</i> | 4400042 |
| <i>Pharmacogenomics Experiments Application Guide</i> | MAN0009612 |
| QuantStudio™ 3 or 5 Real-Time PCR System | |
| <i>QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i> | MAN0010407 |
| <i>QuantStudio™ Design and Analysis Desktop Software User Guide</i> | MAN0010408 |
| QuantStudio™ 6 / QuantStudio™ 7 Flex Real-Time PCR System | |
| <i>QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide</i> | 4489821 |
| <i>QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Getting Started Guide</i> | 4489822 |
| QuantStudio™ 12K Flex Real-Time PCR System | |
| <i>QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide</i> | 4470689 |
| <i>QuantStudio™ 12K Flex Real-Time PCR System: Multi-Well Plates and Array Card Experiments User Guide</i> | 4470050 |
| ViiA™ 7 Real-Time PCR System | |
| <i>Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security</i> | 4442661 |
| <i>Applied Biosystems™ ViiA™ 7 Real-Time PCR System Getting Started Guide</i> | 4441434 |
| 7500/7500 Fast Real-Time PCR System | |
| <i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide</i> | 4347828 |
| <i>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System Getting Started Guide: Relative Standard Curve and Comparative C_t Experiments</i> | 4387783 |
| StepOnePlus™ Real-Time PCR System | |
| <i>Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation Quick Reference Card</i> | 4376783 |
| <i>StepOne™ and StepOnePlus™ Real-Time PCR Systems Installation, Networking and Maintenance User Guide</i> | 4376782 |

| Document | Pub. No. |
|--|----------|
| <i>Applied Biosystems™ StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_t Experiments Getting Started Guide</i> | 4376785 |
| 7900HT Real-Time PCR System | |
| <i>Applied Biosystems™ 7900HT Fast Real-Time PCR System Maintenance and Troubleshooting Guide</i> | 4365542 |
| <i>Applied Biosystems™ 7900HT Fast Real-Time PCR System User Bulletin</i> | 4352533 |

Customer and technical support

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 - Training for many applications and instruments
- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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.

