

PROTOCOL

applied  
biosystems®  
by *life* technologies™

# TaqMan® Mutation Detection Assay

## Competitive Allele-Specific TaqMan® PCR

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*life*  
technologies™

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# How to Use this Guide

## Finding safety information

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**IMPORTANT!** Before using this product, read and understand the information the “Safety” appendix in this document.

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## Understanding user attention words

Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:


**Note:** Provides information that may be of interest or help but is not critical to the use of the product.

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**IMPORTANT!** Provides information that is necessary for proper instrument operation or accurate chemistry kit use.


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 **CAUTION!** Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.


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 **WARNING!** Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

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 **DANGER!** Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

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## Obtaining documents, materials, and SDSs

To obtain...	See...
Documents that are referenced in this Protocol	<a href="#">“Documentation and Support” on page 49</a>
Materials and equipment that are referenced in this Protocol	<ul style="list-style-type: none"><li>• <a href="#">“User-supplied materials” on page 12</a> and/or</li><li>• <a href="#">Appendix A, “Reaction Plates and Accessories” on page 27.</a></li></ul>
The Safety Data Sheet (SDS) for any chemical supplied by Life Technologies	<a href="#">“SDSs” on page 46</a>





# TaqMan<sup>®</sup> Mutation Detection Assays

## Product information

### Purpose of the product

The TaqMan<sup>®</sup> Mutation Detection Assays detect and measure somatic mutations in genes that are associated with cancer. The assays are compatible with different samples types, such as cell lines, FFPE tissue samples, and fresh-frozen tissue samples.

The TaqMan<sup>®</sup> Mutation Detection Assays are powered by castPCR<sup>™</sup> technology, which refers to Competitive Allele-Specific TaqMan<sup>®</sup> PCR. The castPCR<sup>™</sup> technology is highly specific and sensitive, and it can detect and quantitate rare amounts of mutated DNA in a sample that contains large amounts of normal, wild type gDNA.

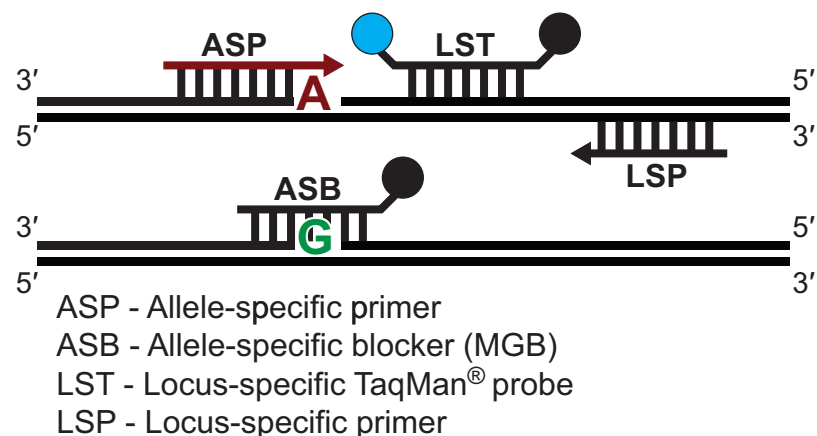
### Mutant Allele Assays

Mutant allele assays target key somatic mutations in oncogene and tumor suppressor genes. All mutation targets are from the comprehensive Sanger COSMIC database. Target selection was based on frequency of occurrence and input from leading cancer researchers.

Each mutant allele assay (see figure, below) detects specific or multiple mutant alleles. Each assay contains:

- an allele-specific primer that detects the mutant allele
- a MGB oligonucleotide blocker suppresses the wild type allele
- a locus specific primer
- a locus specific TaqMan<sup>®</sup> FAM<sup>™</sup> dye-labeled MGB probe

Figure 1 Mutant allele assay using castPCR<sup>™</sup> technology





## Gene reference assays

Gene reference assays detect the genes that the target mutations reside in. They are designed to amplify a mutation-free and polymorphism-free region of the target gene. Each assay contains:

- a locus-specific pair of forward and reverse primers
- a locus specific TaqMan® FAM<sup>TM</sup> dye-labeled MGB probe

**Note:** The first released set of TaqMan® Mutation Detection Assays underwent extensive validation. These validated assays cover a subset of the EGFR, KRAS, and BRAF mutation targets. The mutant allele assays in this set have corresponding wild type allele assays in addition to corresponding gene reference assays. The Calibration  $\Delta C_T$  values, which represent the inherent  $C_T$  difference between mutant allele assays and corresponding reference assays, were determined. This determination enabled quantitative analysis of percent mutation in a sample. Additionally, detection  $\Delta C_T$  cutoff values were predetermined for the validated assay set. These assays are described in more detail in Appendix C.

## Where to find the assays

To view and order TaqMan® Mutation Detection Assays, refer to the product pages at:

[www.lifetechnologies.com/castpcr](http://www.lifetechnologies.com/castpcr)

From this web page, you can search for assays by keyword (e.g. gene symbol or COSMIC identifier) as well as download a TaqMan® Mutation Detection Assay Index file that contains the current assay collection with target and assay information.

## How the assays work

In a mutation detection experiment, a sample of unknown mutation status is run in individual real-time PCRs with one or more assays that target mutant alleles within a gene and the corresponding gene reference assay. After amplification, the  $C_T$  values are determined by the Applied Biosystems® real-time PCR instrument software. Data files containing the sample  $C_T$  values can be exported from the real-time PCR instrument software and imported into a data analysis tool. Life Technologies Mutation Detector<sup>TM</sup> Software is recommended for post-PCR data analysis of mutation detection experiments.

In mutation analysis calculations, the difference between the  $C_T$  value of each mutant allele assay and the  $C_T$  value of the gene reference assay is calculated. This  $\Delta C_T$  value represents the quantity of the specific mutation allele detected within the sample; it is used to determine sample mutation status by comparison to a predetermined detection  $\Delta C_T$  cutoff value.

## Product part numbers, contents, and storage conditions

### Assays

The TaqMan® Mutation Detection Assays collection includes the following inventoried assays:

Product	Part no.	Volume	Contents	Storage conditions
TaqMan® Mutation Detection Assays (mutant allele and wild type allele assays) <sup>†</sup>	4465804	150 µL	A 10X mix that includes: <ul style="list-style-type: none"> <li>• Unlabeled PCR primers</li> <li>• A TaqMan® MGB probe (FAM™ dye-labeled)</li> <li>• An MGB oligonucleotide blocker</li> </ul>	Store at –15°C to –25°C, protected from light. Minimize freeze-thaw cycles.
TaqMan® EGFR Exon 19 Deletions Assay (assay detects 19 mutant alleles)	4465805	150 µL	A 10X mix that includes: <ul style="list-style-type: none"> <li>• Unlabeled PCR primers</li> <li>• A TaqMan® MGB probe (FAM™ dye-labeled)</li> <li>• An MGB oligonucleotide blocker</li> </ul>	
TaqMan® Mutation Detection Reference Assays (gene reference assays)	4465807	150 µL	A 10X mix that includes: <ul style="list-style-type: none"> <li>• Unlabeled PCR primers</li> <li>• A TaqMan® MGB probe (FAM™ dye-labeled)</li> </ul>	

<sup>†</sup> Wild type allele assays are available for a validated subset of the TaqMan Mutation Detection Assays. See Appendix C for more details.

### (Optional) IPC Reagent kit

The TaqMan® Mutation Detection IPC Reagent Kit (sold separately) is preoptimized for use with the TaqMan® Mutation Detection Assays.

The IPC reagents can be duplexed with any TaqMan® Mutation Detection Assay and gDNA sample without compromising the mutation detection assay reaction efficiency. This feature allows the mutation detection assays to detect low copies of target DNA in the presence of a robust PCR-positive signal from the IPC reagents.

Product	Part no.	Contents	Volume	Storage conditions
TaqMan® Mutation Detection IPC Reagent Kit	4467538	<b>50X Exogenous IPC template DNA</b> – A synthetic template that contains the exogenous, non-human DNA target for the assay	200 µL	Store at –15°C to –25°C, protected from light. Minimize freeze-thaw cycles.
		<b>10X Exogenous IPC Mix</b> – A primer-limited TaqMan® assay, designed to amplify an exogenous, non-human DNA target; the mix includes: <ul style="list-style-type: none"> <li>• Two PCR primers</li> <li>• A TAMRA™ probe (VIC® dye-labeled)</li> </ul>	1.0 mL	

## Shipment contents

Each TaqMan® Mutation Detection Assay shipment contains:

- One tube of TaqMan® Mutation Detection Assay mix for each assay ordered
- One Information CD containing the following documents:
  - *Understanding Your Shipment*: A directory of the shipment contents
  - *Data Sheet*: Information about your order
  - *TaqMan® Mutation Detection Assays Product Insert*: A brief overview of the assays
  - *TaqMan® Mutation Detection Assays Protocol*: Procedures for performing experiments with the assays
  - *TaqMan® Mutation Detection Assays Quick Reference Card*: A summary of the Protocol
  - *Assay Information File (AIF)*: The technical details for all assays in the shipment
  - *Certificate of Analysis*: Validation results for your assays
  - *Safety Data Sheet (SDS)*: Safety guidelines

## User-supplied materials

**IMPORTANT!** For the Safety Data Sheet (SDS) of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

### To prepare the samples

For gDNA extraction, we recommend one of the following kits:

Sample type	Product	Source
FFPE tissue samples	RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	Life Technologies AM1975
	QIAamp® DNA FFPE Tissue Kit	Qiagen 56404
Fresh-frozen tissue samples	MELT™ Total Nucleic Acid Isolation System	Life Technologies AM1983
	Gentra Puregene Tissue Kit	Qiagen 158667
Cell lines	PureLink® Genomic DNA Mini Kit	Life Technologies K1820
	Other DNA purification kit	Other major supplier

## To perform the PCR

### Supported real-time PCR systems

The following Applied Biosystems® real-time PCR systems support the TaqMan® Mutation Detection Assays:

Applied Biosystems® real-time PCR system	Block module	Software versions for export to the Mutation Detector™ Software†
7500 system	Standard 96-well Block Module	SDS Software v1.x and v2.x
7500 Fast system	Fast 96-well Block Module	SDS Software v1.x and v2.x
7900HT Fast system	Standard 96-well Block Module, Fast 96-well Block Module, 384-well Block Module	SDS Software v2.x
StepOnePlus® system	Fast 96-well Block Module	StepOne™ Software v2.x
ViiA™ 7 system	Standard 96-well Block Module, Fast 96-well Block Module, 384-well Block Module	ViiA™ 7 Software v1.x
QuantStudio™ 12K Flex system	Standard 96-well Block Module, Fast 96-well Block Module, 384-well Block Module	QuantStudio™ 12K Flex Software v1.x

† For downstream analysis with the Mutation Detector Software: The Mutation Detector Software can import instrument results files (\*.txt or \*.csv files) from Absolute Quantitation or Quantitation-Standard Curve experiments run on the real-time PCR system software versions listed above.

### Consumables

Product	Source
Microcentrifuge tubes, 1.5-mL	Major Laboratory Suppliers (MLS)
Reaction plates and accessories for your real-time PCR system	Life Technologies For part numbers, see <a href="#">Appendix A on page 27</a> .

### Reagents

Product	Source		Volume
TaqMan® Genotyping Master Mix, 2X	Life Technologies	4371355	1-Pack; one 10-mL bottle
		4381656	2-Pack; two 10-mL bottles
		4371357	Single Bulk Pack; one 50-mL bottle
		4381657	Multi Bulk Pack; two 50-mL bottles
Nuclease-free water	MLS		—

## Experiment overview

### Two experiment types

Two types of experiments are required for mutation detection analysis:

1. Detection  $\Delta C_T$  cutoff determination. Run the mutant allele assay and the corresponding gene reference assay on wild type gDNA samples that are from the same sample type as the test sample (e.g. gDNA from FFPE tissue samples). Run at least three different wild type samples and three technical replicates of each sample.  $\Delta C_T$  values are calculated for each sample run with a mutant allele assay - gene reference assay pair. The average  $\Delta C_T$  value for all samples is then calculated and is used to derive the detection  $\Delta C_T$  cutoff value for the mutant allele assay.
2. Mutation detection. Run the test sample with a mutant allele assay(s) and corresponding gene reference assay. The  $\Delta C_T$  value for the mutant allele assay - gene reference assay pair is calculated and this value is compared to the predetermined detection  $\Delta C_T$  cutoff value to determine the sample mutation status.

**Note:** The two experiment types can be run either sequentially or at the same time. All wild type gDNA samples used for determination of  $\Delta C_T$  cutoff values can be run together on one plate or on separate plates; the sample  $\Delta C_T$  values from multiple plates can be combined to generate an assay  $\Delta C_T$  cutoff value. Once the mutant allele assay detection  $\Delta C_T$  cutoff values are determined, these can be used for all subsequent mutation detection experiments run with the same sample type.

For more information on the calculations for each experiment type, see [“How the Mutation Detector™ Software performs the mutation analysis” on page 23](#).

### Experiment steps

1. Run purified gDNA with one or more mutant allele assays and a corresponding gene reference assay. For each real-time PCR, combine the gDNA with:
  - A TaqMan® Mutation Detection Assay. The assay contains two primers and a FAM™ dye-labeled MGB probe to detect a mutant allele or gene reference target. Mutant allele assays also contain an MGB oligonucleotide blocker.
  - TaqMan® Genotyping Master Mix. The master mix contains AmpliTaq Gold® DNA Polymerase, UP (Ultra Pure), dNTPs, and buffer required for the qPCR reactions
  - (Optional) TaqMan® Mutation Detection IPC Reagent Kit. The kit contains an internal positive control (IPC) template, two primers, and a VIC® dye-labeled TAMRA™ probe. You can add the IPC reagents to any mutation detection assay reaction to distinguish true target negatives from PCR failure or inhibition.
2. Run the reactions on a real-time PCR system, using a universal mutation detection thermal-cycling protocol. After the run, the real-time PCR system analysis software determines the  $C_T$  values for the mutation detection assays and (optional) IPC reagent reactions.
3. (Recommended) Export the Results or Results Table from the real-time PCR system software as \*.csv or \*.txt files, then import the files into the Mutation Detector™ Software for downstream analysis.



## Workflow

Prepare the samples ([page 15](#))

1. Extract and purify gDNA ([page 15](#))
2. Quantitate the gDNA ([page 15](#))

Perform the PCR ([page 16](#))

1. Prepare the PCR mix and the PCR plate ([page 18](#))
2. Set up the plate document or experiment and start the run ([page 20](#))

Analyze the data ([page 22](#))

## Prepare the samples

### Extract and purify gDNA

Extract and purify gDNA samples according to your laboratory practices. We recommend the gDNA extraction and purification kits listed below. For gDNA extraction from:

- **Formaldehyde- or paraformaldehyde-fixed, paraffin-embedded (FFPE) tissue samples** – RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE or QIAamp® DNA FFPE Tissue Kit
- **Fresh-frozen tissue samples** – MELT™ Total Nucleic Acid Isolation System or Gentra Puregene Tissue Kit
- **Cell lines** – PureLink® Genomic DNA Mini Kit or kit from other major supplier

### Quantitate the gDNA

Quantitate the gDNA by measuring the UV absorbance ( $A_{260}/A_{280}$ ). Be sure that the human gDNA that you use has an  $A_{260}/A_{280}$  ratio >1.7.

Given that gDNA quantitation by UV absorbance methods may not accurately reflect the concentration of amplifiable gDNA template in some sample types (e.g. FFPE samples), also consider using gene reference assays to assess the amount of functional template in a sample that can be amplified by PCR (for more details, see, [“Sample input range” on page 16](#)).

## Perform the PCR

### Sample input range

Sample	Input range	Recommended amount
gDNA from FFPE tissues	10–50 ng	20 ng
gDNA from fresh-frozen tissues	1–50 ng	20 ng
gDNA from cell lines	1–100 ng	20 ng

The recommended lower limit amount of gDNA input for FFPE tissue samples is higher than that for other types of samples because FFPE tissue samples contain cross-linked, fragmented, and damaged DNA template. The amount of functional template in FFPE tissue gDNA samples is significantly lower than in other types of gDNA samples. The recommended upper limit amount of gDNA input for tissue samples is less than that for cell line samples because PCR inhibitors are typically found in tissue gDNA sample preparations.

The mutant allele assays can detect as low as 0.1% mutated DNA in a background of wild type DNA. To detect:

- 0.1% mutation, use  $\geq 20$  ng of functional gDNA template in the PCR
- 1% mutation, use  $\geq 2$  ng of functional gDNA template in the PCR

Quantitation based on UV absorbance readings may not reflect the concentration of functional gDNA template in a sample that can be amplified by the PCR. As an alternative, you can use the gene reference assays to assess the quantity of template in a sample that can be amplified by the PCR, as follows:

Before you run the mutation detection experiment, run a gene reference assay on the samples, following the procedures in this Protocol. The  $C_T$  value determined for a gene reference assay on a sample should be approximately 18–28 for a 20- $\mu$ L reaction and 17–27 for a 10- $\mu$ L reaction; these ranges correspond to a functional gDNA template input range of approximately 100 ng to 1 ng.

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**IMPORTANT!** For  $\Delta C_T$  cutoff determination, the gene reference assay  $C_T$  values for each wild type gDNA sample used must be similar. If the standard deviation of the  $C_T$  values is large, than an aberrantly small assay  $\Delta C_T$  cutoff value may be calculated, which would effectively decrease the sensitivity of the mutation detection experiment. Additionally, the test sample input amount for mutation detection experiments should be similar to the wild type gDNA sample amount that was used for  $\Delta C_T$  cutoff determination.

For information on mutation analysis calculations, see, [“How the Mutation Detector™ Software performs the mutation analysis” on page 23](#)

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## Recommended reaction types

When setting up the reaction plate, we recommend that you include the following reaction types:

Reaction type	Experiment type	
	Detection $\Delta C_T$ cutoff determination	Mutation detection
Controls	No template controls (NTC) are optional, negative control samples are required (inherent to the experiment), and positive controls are not necessary.	NTCs are optional, negative sample controls are highly recommended, and positive control samples are recommended, if available.
Assays and replicates	To generate accurate detection $\Delta C_T$ cutoff values for a mutant allele assay(s), run the assay and corresponding gene reference assay on at least three wild type gDNA samples and use three technical replicates per sample. The sample type must be the same as the test sample. The same amount of amplifiable DNA should be used for each sample tested.	Run gDNA test samples of unknown mutation status with a mutant allele assay(s) and corresponding gene reference assay. Technical replicates are not required. The same amount of amplifiable DNA should be used for the test sample as was used to establish the detection $\Delta C_T$ cutoff value.
	In order for the Mutation Detector™ Software to compute $\Delta C_T$ cutoff values or sample mutation status, you must run each mutant allele assay, the corresponding gene reference assay, and (if applicable) technical replicates on the same reaction plate with the same sample. For $\Delta C_T$ cutoff determination, the software can combine wild type sample $\Delta C_T$ values from multiple plates to generate an assay $\Delta C_T$ cutoff value.	
(Optional) TaqMan® Mutation Detection IPC Reagents Kit	You can duplex the IPC reagents with any TaqMan® Mutation Detection Assay to distinguish true target negatives from PCR failure or inhibition. See <a href="#">“Analyze the IPC reactions” on page 25</a> .	

**Note:** For  $\Delta C_T$  cutoff determination, the same amount of DNA must be used for each wild type gDNA sample used. For mutation detection experiments, the test sample input amount should be similar to the wild type gDNA sample amount that was used for  $\Delta C_T$  cutoff determination.

## Prepare the PCR mix and the PCR plate

### Detection $\Delta C_T$ cutoff determination experiments

In a detection  $\Delta C_T$  cutoff determination experiment, run three or more wild type gDNA samples, and three technical replicates of each sample, with a mutant allele assay(s) and paired gene reference assay. The same amount of gDNA must be used for each sample. Prepare the PCR mix and the PCR plate as follows:

1. For each sample, calculate the total number of reactions required.
2. Calculate the total volume required each component:  
*volume for 1 reaction × total number of reactions*
3. Include 10% excess volume to account for pipetting errors.

Component	Volume for 1 reaction	
	20 $\mu$ L reaction (96-well plate)	10 $\mu$ L reaction (384-well plate)
TaqMan® Genotyping Master Mix, 2X	10.0 $\mu$ L	5.0 $\mu$ L
Prepared gDNA sample <sup>†</sup>	2.0 – 4.0 $\mu$ L	1.0 – 2.0 $\mu$ L <sup>‡</sup>
(Optional) 50X Exogenous IPC Template DNA	0.4 $\mu$ L	0.2 $\mu$ L
(Optional) 10X Exogenous IPC Mix	2.0 $\mu$ L	1.0 $\mu$ L
Nuclease-free water	Add water to 18.0 $\mu$ L	Add water to 9.0 $\mu$ L
<b>Total volume of super mix</b>	<b>18.0 <math>\mu</math>L</b>	<b>9.0 <math>\mu</math>L</b>
<b>Example: Total volume for 3 technical replicates (includes 10% extra volume for pipetting errors)</b>	<b>59.4 <math>\mu</math>L</b>	<b>29.7 <math>\mu</math>L</b>

<sup>†</sup> We recommend that you input 20 ng of gDNA; the volume of gDNA sample should not be greater than 20% of the total reaction volume.

<sup>‡</sup> For 10- $\mu$ L reactions, we recommend that you use the same amount of gDNA as for 20- $\mu$ L reactions to obtain the same sensitivity of mutation detection.

4. Label a 1.5-mL microcentrifuge tube, add all components to the labeled tube, cap the tube, then vortex the tube briefly to mix the components.
5. Centrifuge the tube briefly to spin down the contents and eliminate air bubbles.
6. For each set of technical replicates, transfer aliquots of super mix to a microcentrifuge tube, then add the TaqMan® Mutation Detection Assay (mutant allele or gene reference assay) to each tube.

If the total reaction volume for one reaction is...	Add the following volumes to the microcentrifuge tube... <sup>†</sup>	
	Super mix	TaqMan® Mutation Detection Assay (10X)
20 $\mu$ L	59.4 $\mu$ L	6.6 $\mu$ L
10 $\mu$ L	29.7 $\mu$ L	3.3 $\mu$ L

<sup>†</sup> The super mix and assay volumes listed are for three technical replicates.



7. Add the appropriate volume of PCR mix to each reaction well of a PCR plate:

PCR plate	Volume of PCR mix per reaction
96-well	20 µL
384-well	10 µL

8. Cover the plate with an optical adhesive film.
9. Centrifuge the plate briefly to spin down the contents and eliminate air bubbles.

**Note:** PCR, and the mutation detection application in particular, is a highly sensitive experimental method. In order to avoid false positive amplifications due to contamination from amplifications previously performed in your lab, please follow the recommended guidelines in [Appendix F, “PCR Good Laboratory Practices,”](#) on page 43

### Mutation detection experiments

In a mutation detection experiment, run the test sample with a mutant allele assay(s) and corresponding gene reference assay. Technical replicates are not required. The amount of test sample gDNA used should be similar to the amount of wild type gDNA sample that was used for your detection  $\Delta C_T$  cutoff determination experiments. Prepare the PCR mix and the PCR plate as follows:

1. For each sample, calculate the total number of reactions required.
2. Calculate the total volume required each component:  
*volume for 1 reaction × total number of reactions*
3. Include 10% extra volume to compensate for pipetting errors.

Component	Volume for 1 reaction	
	20 µL reaction (96-well plate)	10 µL reaction (384-well plate)
TaqMan® Genotyping Master Mix, 2X	10.0 µL	5.0 µL
Prepared gDNA sample <sup>†</sup>	2.0 – 4.0 µL	1.0 – 2.0 µL <sup>‡</sup>
(Optional) 50X Exogenous IPC Template DNA	0.4 µL	0.2 µL
(Optional) 10X Exogenous IPC Mix	2.0 µL	1.0 µL
Nuclease-free water	Add water to 18.0 µL	Add water to 9.0 µL
<b>Total volume of super mix</b>	<b>18.0 µL</b>	<b>9.0 µL</b>

<sup>†</sup> We recommend that you input 20 ng of gDNA; the volume of gDNA sample should not be greater than 20% of the total reaction volume.

<sup>‡</sup> For 10-µL reactions, we recommend that you use the same amount of gDNA as for 20-µL reactions to obtain the same sensitivity of mutation detection.

4. Label a 1.5-mL microcentrifuge tube, add all components to the labeled tube, cap the tube, then vortex the tube briefly to mix the components.
5. Centrifuge the tube briefly to spin down the contents and eliminate air bubbles.

6. Add the appropriate volume of super mix to each reaction well of a PCR plate:

PCR plate	Volume of super mix per reaction
96-well	18.0 µL
384-well	9.0 µL

7. Add the appropriate volume of TaqMan® Mutation Detection Assay (mutant allele or gene reference assay) to each reaction well:

PCR plate	Volume of TaqMan® Mutation Detection Assay (10X) per reaction
96-well	2.0 µL
384-well	1.0 µL

8. Cover the plate with an optical adhesive film.
9. Centrifuge the plate briefly to spin down the contents and eliminate air bubbles.

### Set up the plate document or experiment and start the run

In the real-time PCR system software:

1. Select the experiment type: **Absolute Quantitation** or **Quantitation - Standard Curve**.
2. For each well that contains a reaction, apply a sample name, assay name, and target or detector name. For downstream analysis with the Mutation Detector™ Software, note the following:

Parameter	Comments
Sample Name	If you enter the sample name as <b>NTC</b> , the Mutation Detector™ Software treats the sample as a No Template Control.
	Apply the same sample name to all wells containing the sample and mutation detection assays that will be analyzed together. If the sample name is not identical for each well across the reaction plate, the Mutation Detector™ Software treats these as different samples.
	If you are using technical replicates, apply the same sample name to the wells of each technical replicate group.  The Mutation Detector™ Software combines data from replicate wells only if the wells share the same sample name. If the replicate wells are named differently, the software analyzes the wells as different samples.
Target Name or Detector Name	In order for the Mutation Detector™ Software to correctly analyze the data, you must use TaqMan® assay names. Using TaqMan® assays names ensures that a mutant allele assay will be paired with the appropriate gene reference assay in the analysis. See <a href="#">"Assay Naming Conventions" on page 29</a> .
	If the IPC reagents are duplexed in the reaction, enter <b>IPC</b> as the detector name.

Parameter	Comments
Reporter and Quencher Names	<p>For wells that contain:</p> <ul style="list-style-type: none"> <li>TaqMan® Mutation Detection Assays, FAM™ dye is the reporter and Non Fluorescent or NFQ-MGB is the quencher</li> <li>IPC reagents (from the TaqMan® Mutation Detection IPC Reagent Kit), VIC® dye is the reporter and TAMRA™ dye is the quencher</li> </ul> <p><b>Note:</b> Instrument results files exported from the 7500 SDS v1.X software do not contain a Reporter column with dye names. In this case, the Mutation Detector™ Software uses the assay name (from the Detector/Target column) to determine the dye name. If the assay name is <i>IPC</i>, the software assumes that the reporter is VIC® dye; for all other assay names, the software assumes that the reporter is FAM™ dye.</p>

3. Enter sample quantity values in the real-time PCR system software:
  - a. Select **Standard** as the task for each well of interest.
  - b. Enter a numeric value. We recommend that the numeric values you enter are relevant to the ng amount of gDNA or copies of DNA input.

For downstream analysis with the Mutation Detector™ Software, note the following:

Parameter	Comments
Amount of sample DNA	For all mutation detection assay results that will be used to calculate a detection $\Delta C_T$ cutoff value or mutation status, load the same amount of sample DNA into the wells. If the sample quantity is not specified in the real-time PCR system software, then the Mutation Detector™ Software assumes that the sample amounts in each well are equivalent.
Sample quantity value	The sample quantity value must be equal and must be provided in the real-time PCR system software for all samples that will be analyzed together if you are calculating calibration $\Delta C_T$ values in real-time (comparing positive control sample $C_T$ values between a mutant allele assay and a corresponding gene reference assay). For more information, see, <a href="#">Appendix D, "Calibration DCT values," on page 35</a> .

**Note:** For  $\Delta C_T$  cutoff determination, load the same amount of amplifiable gDNA for each wild type sample used. The different wild type gDNA samples used can be run on one or more plates; the software can combine sample  $\Delta C_T$  values across plates to generate detection  $\Delta C_T$  cutoff values.

4. Set the following thermal-cycling conditions:

- Run mode – **Standard**
- Sample volume – **10 µL** (384-well plates) or **20 µL** (96-well plates)
- Thermal-cycling profile – See the table below

Stage	Temp.	Time (mm:ss)	Cycles	Data collection
1	95°C	10:00	1	No
2	92°C	00:15	5	No
	58°C	01:00		No
3	92°C	00:15	40	No
	60°C	01:00		FAM™ or VIC® dye†

† FAM dye is the reporter for TaqMan Mutation Detection Assays; VIC dye is the reporter for the IPC reagents (from the TaqMan Mutation Detection IPC Reagent Kit).

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

**For more information**

For more information on running a PCR experiment, refer to the appropriate Getting Started Guide for your instrument. (For information on obtaining documents, see [“Documentation and Support” on page 49.](#))

## Analyze the data

**Analysis steps**

For detailed analysis procedures, refer to the *Mutation Detector™ Software User Guide*. (For information on obtaining documents, see [“Documentation and Support” on page 49.](#))

Briefly, the analysis steps are:

1. Analyze the data in the real-time PCR system software, using the following analysis settings:
  - Manual C<sub>T</sub> (threshold cycle): **0.2**
  - Automatic Baseline: **On**

The real-time PCR system software determines the C<sub>T</sub> values for the mutation detection assays and (optional) IPC reagent reactions.

2. View the amplification plots and/or C<sub>T</sub> values for all reaction wells as follows:

Reaction type	What to look for
Samples tested with gene reference assays (FAM™ dye signal)	Verify that the amplification curves have a distinct, linear amplification phase and that the C <sub>T</sub> values are within a range of ~18 to 28 for a 20-µL reaction and ~17 to 27 for a 10-µL reaction.
Samples tested with mutant allele assays (FAM™ dye signal)	Review the amplification curves and C <sub>T</sub> values. The presence or absence of a distinct, linear amplification phase and C <sub>T</sub> values depend on the amount of mutant allele present in the sample.



Reaction type	What to look for
Positive control samples	Verify that the amplification curves have a distinct, linear amplification phase and that the $C_T$ values are within the expected range for the quantity of target present in the sample.  <b>Note:</b> Positive control samples that are used to calculate calibration $\Delta C_T$ values must contain 100% target sequence for the mutation detection assay that is run with the positive control.
Negative control samples	Verify that the negative control samples <i>either</i> do not amplify <i>or</i> have very high $C_T$ values.
No template control (NTC) samples	Verify that the NTC samples do not amplify.
Technical replicates	Verify that the $C_T$ values are similar between replicates.  <b>Note:</b> Some variance is expected between replicates for samples that contain low amounts of target allele and have high $C_T$ values.
(If using) IPC reagents, from the TaqMan® Mutation Detection IPC Reagent Kit	Verify that the amplification curves (VIC® dye signal) in all samples have a distinct, linear amplification phase and that the $C_T$ values are similar for all wells that contain the same sample

**Note:** Some mutant allele assays are expected to give low level non-specific amplification of wild type gDNA samples. The TaqMan® Mutation Detection Assay Index file (download from: [www.lifetechnologies.com/castpcr](http://www.lifetechnologies.com/castpcr)) provides off-target amplification  $C_T$  values determined for each mutant allele assay that can be used to evaluate an assay's performance. Note that the off-target  $C_T$  value may differ for different sample types.

- Export the Results or Results Table from the real-time PCR system software as a \*.csv or \*.txt file.
- Import the \*.csv or \*.txt file(s) into the Mutation Detector™ Software. The Mutation Detector Software can:
  - Calculate detection  $\Delta C_T$  cutoff values
  - Determine the presence or absence of a mutation in a sample *and*
  - Quantitate the percent mutation in a sample (when assay calibration values are available)

## How the Mutation Detector™ Software performs the mutation analysis

### Determine the detection $\Delta C_T$ cutoff value

In a typical detection  $\Delta C_T$  cutoff determination experiment, three or more wild type gDNA samples, and three technical replicates of each sample, are run with a mutant allele assay and paired gene reference assay. To perform the real-time calculations for the detection  $\Delta C_T$  cutoff values, the Mutation Detector™ Software:

- Calculates the  $\Delta C_T$  value between amplification reactions for the mutant allele assay (negative control) and the gene reference assay, depending on whether or not a calibration  $\Delta C_T$  value is available for the assay pair, as follows:

$$\Delta C_T = C_{T(\text{mutant allele assay negative control})} - C_{T(\text{gene reference assay})}$$

**or**

$$\text{Normalized } \Delta C_T = [C_{T(\text{mutant allele assay negative control})} - C_{T(\text{gene reference assay})}] - \text{Calibration } \Delta C_T$$

**where:** The calibration  $\Delta C_T$  value is the inherent  $C_T$  difference between the mutant allele assay and the corresponding gene reference assay. These values are currently available for a subset of the mutation detection assays. See, [Appendix C, “Validated Assay Set,” on page 31](#) and [Appendix D, “Calibration DCT values,” on page 35](#).

2. Averages the  $\Delta C_T$  value or normalized  $\Delta C_T$  value from each sample.
3. Calculates the detection  $\Delta C_T$  cutoff value, as follows:

*Detection  $\Delta C_T$  cutoff = Average  $\Delta C_T - (3 \times \text{the standard deviation or } 2C_T, \text{ use whichever one is greater})$*

**Note:** We recommend adjusting any detection  $\Delta C_T$  cutoff values that are greater than 9.96 to 9.96  $\Delta C_T$ , which is equivalent to a 0.1% detection cutoff.

Please refer to the *Mutation Detector™ Software User Guide* (Part no. 4467102) for more information on calculating detection  $\Delta C_T$  cutoff values in real time and using these values in mutation detection analysis.

### Determine the mutation status of a sample

The Mutation Detector™ Software determines the mutation status of a sample by first calculating the  $\Delta C_T$  value between amplification reactions for a mutant allele assay and a corresponding gene reference assay, as follows, depending on whether or not a calibration  $\Delta C_T$  value is available for the assay pair.

$$\Delta C_T = C_{T(\text{mutant allele assay})} - C_{T(\text{gene reference assay})}$$

or

$$\text{Normalized } \Delta C_T = [C_{T(\text{mutant allele assay})} - C_{T(\text{gene reference assay})}] - \text{Calibration } \Delta C_T$$

**where:** The calibration  $\Delta C_T$  value is the inherent  $C_T$  difference between the mutant allele assay and the corresponding reference assay. These values are currently available for a subset of the mutation detection assays. For more information, see, [Appendix C, “Validated Assay Set,” on page 31](#) and [Appendix D, “Determining Calibration DCT values,” on page 35](#).

The software then compares the  $\Delta C_T$  value to a predetermined detection  $\Delta C_T$  cutoff value to determine if a mutation is detected within the sample:

If the $\Delta C_T$ value is...	Then...
Greater than the detection $\Delta C_T$ cutoff value	The software classifies the gDNA sample as <b>mutation not detected</b> . The sample is either mutation negative, or below the limit of detection for the TaqMan® Mutation Detection Assays.
Less than the detection $\Delta C_T$ cutoff value	The software classifies the gDNA sample as <b>mutation detected</b> , and calculates the percent mutation in the sample, if the calibration $\Delta C_T$ value for the assay pair is available.





## Determine the percent mutation

For assay pairs having calibration  $\Delta C_T$  values, quantitative mutation analysis is possible. The Mutation Detector™ Software determines the percent mutation present in a mutation-positive sample based on the normalized  $\Delta C_T$  value between amplification reactions for a mutant allele assay and a corresponding gene reference assay using the following calculation:

$$\% \text{mutation} = [1/2^{\Delta C_T} \div (1/2^{\Delta C_T} + 1)] \times 100\%$$

**Note:** The software does not calculate a percent mutation for assays that do not have calibration  $\Delta C_T$  values or that have  $\Delta C_T$  results greater than the detection  $\Delta C_T$  cutoff value.

See, [Appendix D, “Determining Calibration DCT values” on page 35.](#)

## Mutant allele assay evaluation and the TaqMan® Mutation Detection Assay Index

To aid evaluation of mutant allele assay performance, sensitivity (% mutations) and off-target amplification  $C_T$  values have been provided for each mutant allele assay in the TaqMan® Mutation Detection Assay Index file, which can be downloaded from: [www.lifetechnologies.com/castpcr](http://www.lifetechnologies.com/castpcr). Life Technologies determined the sensitivity of each mutant allele assay by running these on samples consisting of 10, 25 or 100 copies of mutant allele plasmid DNA spiked into  $1 \times 10^4$  copies (30 ng) of wild type cell line gDNA, which corresponds to samples containing 0.1%, 0.25%, or 1.0% mutation, respectively. The average off-target amplification  $C_T$  value was determined by running each assay on 30 ng ( $10^4$  copies) of multiple wild type cell line gDNA samples. One can use the sensitivity value to understand the detection limit of each assay. The off-target amplification  $C_T$  value can help one to understand how much off-target amplification is expected for each assay when tested on wild type gDNA samples. Note that these values were determined using cell line gDNA samples; the actual values for FFPE samples or other sample types may differ, yet the provided values can still be used as a guideline for individual assay performance.

## Analyze the IPC reactions

If you included IPC reagents in your reactions (from the TaqMan® Mutation Detection IPC Reagent Kit), the Mutation Detector™ Software simultaneously analyzes the  $C_T$  values in the reaction well for the mutation detection assay (FAM™ dye) and the IPC reaction (VIC® dye).

The software evaluates the IPC reaction results for each well as shown in the table below. In this example, the default limit  $C_T$  values of 30 are used for the IPC VIC dye signal and the mutation detection assay FAM™ dye signal.

IPC (VIC® dye) $C_T$ value	Mutation (FAM™ dye) $C_T$ value	Result
<30	<30	PCR is valid
<30	>30 or Undetermined	PCR is valid
>30 or Undetermined	<30	PCR is valid
>30 or Undetermined	>30 or Undetermined	PCR failure or inhibition

The IPC reaction may fail to generate a  $C_T$  value less than 30 when the mutation detection assay reaction generates a low  $C_T$  value, corresponding to a large quantity of target present in the sample. This failure is acceptable because the IPC reaction result is intended to evaluate PCR failure or inhibition in a reaction.

If both the mutation detection assay and IPC reaction results are negative in a well, the PCR did not occur; in this instance, the mutation-negative result is not valid. You should repeat the reaction and/or perform further troubleshooting.



# Reaction Plates and Accessories

The table below lists the reaction plates and accessories available for Applied Biosystems® real-time PCR systems.

Real-time PCR system	Reaction plates and accessories
7500 system, Standard 96-well Block Module	<ul style="list-style-type: none"><li>• MicroAmp® Optical 96-Well Reaction Plate with Barcode:<ul style="list-style-type: none"><li>– 500 plates (PN 4326659)</li><li>– 20 plates (PN 4306737)</li></ul></li><li>• MicroAmp® Optical Adhesive Film, 100 films (PN 4311971)</li><li>• MicroAmp® Optical Film Compression Pad, 5 pads (PN 4312639)</li><li>• MicroAmp® Optical 8-Cap Strip, 300 strips (PN 4323032)</li></ul>
7500 Fast system, Fast 96-well Block Module	<ul style="list-style-type: none"><li>• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode:<ul style="list-style-type: none"><li>– 200 plates (PN 4366932)</li><li>– 20 plates (PN 4346906)</li></ul></li><li>• MicroAmp® Optical Adhesive Film, 100 films (PN 4311971)</li><li>• MicroAmp® Optical 8-Cap Strip, 300 strips (PN 4323032)</li></ul>
7900HT/7900HT Fast system, 384-Well Block Module	<ul style="list-style-type: none"><li>• MicroAmp® Optical 384-Well Reaction Plate with Barcode:<ul style="list-style-type: none"><li>– 1000 plates (PN 4343814)</li><li>– 500 plates (PN 4326270)</li><li>– 50 plates (PN 4309849)</li></ul></li><li>• MicroAmp® Optical 384-Well Reaction Plate, 1000 plates (PN 4343370)</li><li>• MicroAmp® Optical Adhesive Film, 100 films (PN 4311971)</li></ul>
7900HT/7900HT Fast system, Standard 96-Well Block Module	<ul style="list-style-type: none"><li>• MicroAmp® Optical 96-Well Reaction Plate with Barcode:<ul style="list-style-type: none"><li>– 500 plates (PN 4326659)</li><li>– 20 plates (PN 4306737)</li></ul></li><li>• MicroAmp® Optical Adhesive Film, 100 films (PN 4311971)</li><li>• MicroAmp® Optical Film Compression Pad, 5 pads (PN 4312639)</li><li>• MicroAmp® Optical 8-Cap Strip, 300 strips (PN 4323032)</li><li>• MicroAmp® Snap-On Optical Film Compression Pad, for use with the automation accessory (PN 4333292)</li></ul>
7900HT/7900HT Fast system, Fast 96-Well Block Module	<ul style="list-style-type: none"><li>• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode:<ul style="list-style-type: none"><li>– 200 plates (PN 4366932)</li><li>– 20 plates (PN 4346906)</li></ul></li><li>• MicroAmp® Optical Adhesive Film, 100 films (PN 4311971)</li></ul>

Real-time PCR system	Reaction plates and accessories
ViiA™ 7 system or QuantStudio™ 12K Flex system 384-Well Block Module	<ul style="list-style-type: none"> <li>• MicroAmp® Optical 384-Well Reaction Plate with Barcode: <ul style="list-style-type: none"> <li>– 1000 plates (PN 4343814)</li> <li>– 500 plates (PN 4326270)</li> <li>– 50 plates (PN 4309849)</li> </ul> </li> <li>• MicroAmp® Optical 384-Well Reaction Plate, 1000 plates (PN 4343370)</li> <li>• MicroAmp® Optical Adhesive Film, 100 films (PN 4311971)</li> </ul>
ViiA™ 7 system or QuantStudio™ 12K Flex system Standard 96-Well Block Module	<ul style="list-style-type: none"> <li>• MicroAmp® Optical 96-Well Reaction Plate with Barcode: <ul style="list-style-type: none"> <li>– 500 plates (PN 4326659)</li> <li>– 20 plates (PN 4306737)</li> </ul> </li> <li>• MicroAmp® Optical Adhesive Film, 100 films (PN 4311971)</li> <li>• MicroAmp® Optical Film Compression Pad, 5 pads (PN 4312639)</li> <li>• MicroAmp® Optical 8-Cap Strip, 300 strips (PN 4323032)</li> </ul>
ViiA™ 7 system or QuantStudio™ 12K Flex system Fast 96-Well Block Module	<ul style="list-style-type: none"> <li>• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode: <ul style="list-style-type: none"> <li>– 200 plates (PN 4366932)</li> <li>– 20 plates (PN 4346906)</li> </ul> </li> <li>• MicroAmp® Optical Adhesive Film, 100 films (PN 4311971)</li> </ul>
StepOnePlus® system	<ul style="list-style-type: none"> <li>• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode: <ul style="list-style-type: none"> <li>– 200 plates (PN 4366932)</li> <li>– 20 plates (PN 4346906)</li> </ul> </li> <li>• MicroAmp® Optical Adhesive Film, 100 films (PN 4311971)</li> </ul>



# Assay Naming Conventions

## Assay Naming Conventions

The naming conventions for the TaqMan® Mutation Detection Assays are described in the table below.

For...	The assay name contains the...	Example
Mutant allele assays	<ul style="list-style-type: none"><li>• Gene symbol</li><li>• COSMIC ID<sup>‡</sup></li><li>• Suffix <i>_mu</i></li></ul>	KRAS_517_mu
Wild type allele assays <sup>†</sup>	<ul style="list-style-type: none"><li>• Gene symbol</li><li>• COSMIC ID<sup>‡</sup></li><li>• Suffix <i>_wt</i></li></ul>	KRAS_517_wt
Gene reference assays	<ul style="list-style-type: none"><li>• Gene symbol</li><li>• Suffix <i>_rf</i></li></ul>	KRAS_rf

<sup>†</sup> Wild type allele assays are available for a validated subset of the Taqman Mutation Detection Assays. See Appendix C for more details.

<sup>‡</sup> The COSMIC ID is from the Catalogue of Somatic Mutations in Cancer ([www.sanger.ac.uk/genetics/CGP/cosmic/](http://www.sanger.ac.uk/genetics/CGP/cosmic/)).

The Mutation Detector™ Software uses the TaqMan® assay names to pair the mutant allele assays with corresponding wild type allele assays or gene reference assays for mutation detection analysis, and to associate the assays with assay-specific attributes (for example, calibration  $\Delta C_T$  values and detection  $\Delta C_T$  cutoff values).





# Validated Assay Set

This appendix covers:

- About the TaqMan® Mutation Detection Assays validated assay set . . . . . 31
- Mutation analysis using mutant allele assays run with wild type allele assays. 33

## About the TaqMan® Mutation Detection Assays validated assay set

The first set of TaqMan® Mutation Detection Assays underwent extensive validation. This set of validated assays covers 14 KRAS targets, 29 EGFR targets, and the BRAF V600E mutation targets and is referred to as the validated assay set. The main difference between the validated assays and other TaqMan® Mutation Detection Assays is the inclusion of the following attributes in the validated assays:

1. The mutant allele assays have corresponding wild type allele assays in addition to corresponding gene reference assays. These assays enable more accurate quantitation of the percent mutation in a sample.
2. The inherent  $C_T$  difference between mutant allele assays and the corresponding gene reference assay or wild type allele assay have been predetermined, enabling quantitative analysis of percent mutation in a sample.
3. Detection  $\Delta C_T$  cutoff values have been predetermined for the validated assay set.

### Wild type allele assays

Wild type allele assays for corresponding mutant allele assays use castPCR™ technology. Each wild type allele assay contains:

- an allele-specific primer that detects the wild type allele
- a MGB oligonucleotide blocker that suppresses the mutant allele
- a locus specific primer
- a locus specific TaqMan® FAM™ dye-labeled MGB probe

Validated mutant allele assays can be paired with corresponding wild type allele assays, versus gene reference assays, in a mutation detection experiment for more highly accurate quantitation of the percent mutation in a sample. The basic mutation detection protocol can be used; however, it is recommended that three technical replicates are run for highest quantitation accuracy.

### Calibration $\Delta C_T$ values

The calibration  $\Delta C_T$  value is the inherent  $C_T$  difference between a mutant allele assay and a wild type allele assay or a gene reference assay. This difference is determined by amplifying equal amounts of DNA templates containing 100% mutant allele (for the mutant allele assay) and 100% wild type DNA (for the wild type allele assay or gene

reference assay). Life Technologies determined the calibration  $\Delta C_T$  values for the validated assay set in experiments using  $10^4$  copies of synthetic DNA templates. For more information on Calibration  $\Delta C_T$  values, see [Appendix D, “Determining Calibration  \$\Delta C\_T\$  values,” on page 35.](#)

## Detection $\Delta C_T$ cutoff values

The detection  $\Delta C_T$  cutoff value is used to determine the lower limit amount of mutation in a sample that the mutant allele assay can detect. The detection  $\Delta C_T$  cutoff values were determined for the validated assay set in studies where each mutant allele assay was run with its corresponding wild type allele assay and gene reference assay on multiple genomic DNA (gDNA) samples isolated from wild type cell line samples. The  $\Delta C_T$  value between amplification reactions for the mutant allele assay and the corresponding wild type allele assay or gene reference assay was used to calculate the detection  $\Delta C_T$  cutoff values (see [page 33](#) for the equations used). Calculated detection  $\Delta C_T$  cutoff values were close in value for each mutant allele assay run with corresponding wild type allele assays and gene reference assays; the smaller calculated detection  $\Delta C_T$  cutoff value was used for the mutant allele assay. All detection  $\Delta C_T$  cutoff values greater than 9.96 were adjusted to 9.96, equivalent to a 0.1% detection cutoff.

To confirm that the predetermined mutant allele assay 0.1% sensitivity value was relevant to mutation detection analysis in gDNA isolated from cellular and FFPE samples, Applied Biosystems conducted TaqMan® Mutation Detection Assays experiments with:

- **Cellular gDNA samples** – 10 copies of plasmid DNAs carrying mutant alleles spiked into 30 ng (equivalent to  $10^4$  copies) of normal cell-line gDNA. In these studies, the mutant allele assay sensitivity was as low as 0.1%.
- **gDNA isolated from FFPE samples** – Limited testing done with available gDNA isolated from FFPE samples demonstrated equivalent levels of assay sensitivity.

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**IMPORTANT!** Genomic DNA extracted from alternate sample types and preparation methods may have different cutoff values. We recommend that you determine the detection  $\Delta C_T$  cutoff values for your specific sample types and preparation methods (see [page 33](#)).

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**Note:** The predetermined calibration  $\Delta C_T$  values and detection  $\Delta C_T$  cutoff values are provided in the TaqMan® Mutation Detection Assay Index file, which can be downloaded from: [www.lifetechnologies.com/castpcr](http://www.lifetechnologies.com/castpcr). They are also found in the ‘TMDA\_assay\_attribute\_file.txt’ file which is located in the Mutation Detector™ Software installation folder and in the software Assay Attributes table when these assays are present in a mutation analysis.



## Mutation analysis using mutant allele assays run with wild type allele assays

When mutant allele assays are run with wild type allele assays in a mutation detection experiment, the following variations in the mutation analysis calculation will be performed by Mutation Detector™ Software.

### Determines the detection $\Delta C_T$ cutoff value

To determine detection  $\Delta C_T$  cutoff values for your own sample type (recommended), the basic protocol for detection  $\Delta C_T$  cutoff values determination on page 23 should be followed, with the exception that each mutant allele assay will be paired with its corresponding wild type allele assay. After sample amplification, the  $\Delta C_T$  value between amplification reactions for the mutant allele assay and the wild type allele assay will be calculated as follows:

$$\text{Normalized } \Delta C_T = [C_{T(\text{mutant allele assay negative control})} - C_{T(\text{wild type allele assay})}] - \text{Calibration } \Delta C_T$$

Then calculate the  $\Delta C_T$  cutoff value, as follows:

$$\text{Detection } \Delta C_T \text{ cutoff} = \text{Average normalized } \Delta C_T - [3 \times \text{the standard deviation or } 2 C_T, \text{ whichever is greater}]$$

### Determines the mutation status

The Mutation Detector Software determines the mutation status of a sample by first calculating the  $\Delta C_T$  value between amplification reactions for a mutant allele assay and a corresponding wild type allele assay as follows:

$$\text{Normalized } \Delta C_T = [C_{T(\text{mutant allele assay})} - C_{T(\text{wild type allele assay})}] - \text{Calibration } \Delta C_T$$

The software then compares the  $\Delta C_T$  value to a predetermined detection  $\Delta C_T$  cutoff value to determine if a mutation is detected within the sample. If a sample is mutation positive, then the quantity of mutated DNA present relative to the total quantity of DNA is calculated.

### Determines the percent mutation

The Mutation Detector™ Software determines the percent mutation present in a mutation-positive sample based on the normalized  $\Delta C_T$  value between amplification reactions for a mutant allele assay and a corresponding wild type allele assay using the following calculation:

$$\% \text{mutation} = [1/2^{\Delta C_T} \div (1/2^{\Delta C_T} + 1)] \times 100\%$$

For more information on the validated assay set, please see the Application Note *Accurate and sensitive mutation detection and quantitation using TaqMan® Mutation Detection Assays for disease research* (Part no. CO31540 01011), which can be downloaded from [www.LifeTechnologies.com](http://www.LifeTechnologies.com).



## **Appendix C** Validated Assay Set

*Mutation analysis using mutant allele assays run with wild type allele assays*



# Determining Calibration $\Delta C_T$ values

## Calibration $\Delta C_T$ values

The calibration  $\Delta C_T$  value is the  $C_T$  difference between the mutant allele assay and the corresponding gene reference assay when assays are run on the same amount of pure target DNA for the assay. Calibration  $\Delta C_T$  values for an assay pair in an analysis are required for quantitation of the percent mutation in a sample. If calibration  $\Delta C_T$  values are not available, then only the qualitative yes/no mutation status can be determined.

Theoretically, if assays have 100% amplification efficiency, then mutant allele assays and the corresponding gene reference assays should generate the same  $C_T$  value for the same amount of on-target templates. In reality, different assays have different amplification efficiencies; therefore, they do not always generate the same  $C_T$  values for equal quantities of DNA. This difference is determined by amplifying equal amounts of DNA templates containing 100% mutant allele (for the mutant allele assay) and 100% wild type DNA (for the gene reference assay). Life Technologies calculated calibration  $\Delta C_T$  values for the validated subset of the TaqMan® Mutation Detection Assays (see App. C) using  $10^4$  copies of synthetic plasmids as positive control templates.

Calibration  $\Delta C_T$  values reflect the inherent difference in the mutant allele assay and the corresponding gene reference assay amplification efficiencies. The calibration  $\Delta C_T$  values theoretically should not vary significantly between different sample types, thus they can be determined using plasmid DNA templates in well-controlled experiments. These values can also be generated for your own sample types, if desired, though it may be difficult to acquire the requisite 100% mutant allele samples. If you are interested in generating calibration  $\Delta C_T$  values, see "Guidelines for generating calculated values" below.

### Guidelines for generating calculated values

To generate meaningful calibration  $\Delta C_T$  values, we recommend the following:

- Run at least three technical replicates per sample
- Samples must be of the highest quality and must be accurately quantified for functional template
- The mutant allele assay positive control is a DNA sample containing 100% mutant allele for the target assay
- The corresponding gene reference assay positive control is a DNA sample containing 100% wild type gene target
- The positive control samples must contain the same amount of DNA and must be of the same sample type

**How the Mutation Detector Software performs the calculations**

The Mutation Detector Software calculates calibration  $\Delta C_T$  values in real-time for paired mutant allele assays and corresponding gene reference assays by:

1. Averaging the  $C_T$  values from the technical replicates
2. Calculating the efficiency difference between amplification reactions for the mutant allele assay and gene reference assay as follows:

$$\text{Calibration } \Delta C_T = C_{T(\text{mutant allele assay positive control})} - C_{T(\text{gene reference assay positive control})}$$

Please refer to the Mutation Detector Software User Guide for more information on calculating calibration  $\Delta C_T$  values in real time and using these values in mutation detection analysis.



# Troubleshooting and Frequently Asked Questions

## Troubleshooting table

Observation	Possible Cause	Recommended Action
Amplification in No Template Control (NTC) wells	Random contamination of wells by template when loading PCR plate	Follow the lab practices in <a href="#">Appendix F, "PCR Good Laboratory Practices" on page 43</a> .
	Reagent contamination with template	
Amplification of negative control, wild type gDNA samples by mutant allele assays	Random or reagent contamination with mutant allele template	Follow the lab practices in <a href="#">Appendix F, "PCR Good Laboratory Practices" on page 43</a> .
	Some mutant allele assays, designed to difficult target sequences, are expected to give low level non-specific amplification of wild type samples.	Refer to the TaqMan® Mutation Detection Assay Index file, which provides Life Technologies off-target amplification C <sub>T</sub> values for each mutant allele assay run on 30 ng wild type cell line gDNA samples. This information can be used to evaluate assay performance. Note that the off-target C <sub>T</sub> value may differ for different sample types.

Observation	Possible Cause	Recommended Action
Determined detection $\Delta C_T$ cutoff value is small and/or assay sensitivity is low	High Ct variation and standard deviation values for wild type gDNA samples run with gene reference assays	<p>The same amount of amplifiable DNA template must be used for each wild type DNA sample used to determine a <math>\Delta C_T</math> cutoff value.</p> <ul style="list-style-type: none"> <li>• Check your sample preparation and quantification procedures.</li> <li>• For difficult sample types (e.g. FFPE tissues), consider first running samples with the gene reference assay to check for the amount of amplifiable template present before running the <math>\Delta C_T</math> cutoff determination experiment.</li> <li>• Consider removing outlier wild type samples from the <math>\Delta C_T</math> cutoff determination experiment.</li> </ul>
	Some mutant allele assays, designed to difficult target sequences, are expected to give low level non-specific amplification of wild type samples and be less sensitive than others (e.g. may detect down to 1% versus 0.1% mutation).	<p>Refer to the TaqMan® Mutation Detection Assay Index file, which provides Life Technologies sensitivity values (% mutation) for each mutant allele assay determined by spiking experiments. This information can be used to evaluate assay performance. Note that the sensitivity values may differ for different sample types and amount of sample loaded.</p>

## Frequently Asked Questions

### 1. Why do different mutant allele assays have different sensitivities or off-target amplification values?

Mutant allele assay performance is dependent on the nature of the mutation and the flanking genomic DNA context sequences at that particular locus. Mutant allele assays that detect a transversion point mutation (a purine to pyrimidine or a pyrimidine to purine change [C/T ↔ A/G]) usually have high sensitivity and minimal or no off-target amplification of wild type gDNA. Mutant allele assays that detect transition mutations (a purine to purine [A ↔ G] or pyrimidine to pyrimidine [C ↔ T] change) usually have relatively lower assay sensitivity and higher off-target amplification, especially for mutations that occur within GC-rich regions. Additionally, assays that detect difficult, repetitive InDel sequences may also have relatively lower assay sensitivity and higher off-target amplification.

To aide your evaluation of mutant allele assay performance, Life Technologies has determined the sensitivity (% mutation) and average off-target amplification  $C_T$  values for each mutant allele assay; these are provided in the TaqMan® Mutation Detection Assay Index file, which can be downloaded from:

[www.lifetechnologies.com/castpcr](http://www.lifetechnologies.com/castpcr).

### 2. How were the mutant allele assay sensitivity and off-target $C_T$ amplification values in the TaqMan® Mutation Detection Assay Index file generated and how should this information be used?

To aid evaluation of mutant allele assay performance, sensitivity (% mutations) and off-target amplification  $C_T$  values have been provided for each mutant allele assay in the TaqMan® Mutation Detection Assay Index file, which can be downloaded from: [www.lifetechnologies.com/castpcr](http://www.lifetechnologies.com/castpcr). Life Technologies determined the sensitivity of each mutant allele assay by running these on samples consisting of 10, 25 or 100 copies of mutant allele plasmid DNA spiked into  $1 \times 10^4$  copies (30 ng) of wild type cell line gDNA, which corresponds to samples containing 0.1%, 0.25%, or 1.0% mutation, respectively. The average off-target amplification  $C_T$  value was determined by running each assay on 30 ng (=  $10^4$  copies) of multiple wild type cell line gDNA samples.

One can use the sensitivity value to understand the detection limit of each assay. The off-target amplification  $C_T$  value can help one to understand how much off-target amplification is expected for each assay when tested on wild type gDNA samples. Note that these values were determined using cell line gDNA samples; the actual values for FFPE samples or other sample types may differ, yet the provided values can still be used as a guideline for individual assay performance.

### 3. How can one best determine the quality and quantity of FFPE tissue samples?

FFPE tissue samples should be prepared using a high quality sample preparation method, as recommended in the protocol. Sample concentration can initially be estimated by a UV absorbance method. However, given that FFPE tissue samples contain cross-linked, fragmented, and damaged DNA template, the amount of functional template in FFPE tissue gDNA samples is significantly lower than in other types of gDNA samples; quantitation based on UV absorbance readings may not reflect the amount of template that can be amplified. As an alternative, you can use the gene reference assays to assess the quantity of template in a sample that can be amplified by the PCR. Life Technologies recommends that the  $C_T$  value determined for a gene reference assay on a sample should be approximately 18–28 for a 20- $\mu$ L reaction and 17–27 for a 10- $\mu$ L reaction.

It is very important to qualify the quality and quantity of your samples before running mutation analysis experiments. Sample DNA quality can affect the performance of the TaqMan® Mutation Detection Assays, and use of low quality samples can compromise the mutation analysis results, particularly when mutant alleles are present at a lower percentage in a sample.

### 4. How can one determine mutant allele assay detection $\Delta C_T$ cutoff values for FFPE sample types when wild type FFPE tissue gDNA samples are difficult to obtain?

To establish detection  $\Delta C_T$  cutoff values, Life Technologies highly recommends running a mutant allele assay and corresponding gene reference assay with at least 3 wild type gDNA samples of the same sample type as the test samples, and to use 3 replicates of each sample. If you are using FFPE tissue gDNA samples and it is difficult to obtain enough corresponding wild type gDNA samples for this analysis, consider using one of the following workarounds:

- Determine the detection  $\Delta C_T$  cutoff value using test samples to substitute for wild type samples. This method is recommended only for experiments having a large number of samples, and assumes that for a specific locus, the mutation only occurs in a small percentage of tested samples. The calculated  $\Delta C_T$  cutoff value can be used to represent the background amplification of mutant allele assays on wild type samples.
- Use a set detection  $\Delta C_T$  cutoff value of 6.67 corresponding to 1% sensitivity. This method is recommended only for experiments having a small number of samples, where the required detection limit is > 1% mutation. All TaqMan® Mutation Detection Assays can detect at least 1% mutation, as demonstrated in experiments using synthetic mutant allele DNA templates spiked into 30 ng cell line gDNA.

**5. Why does the calculation for the detection  $\Delta C_T$  cutoff value include a subtraction of 3 times the standard deviation or 2  $C_T$  values?**

The detection  $\Delta C_T$  cutoff value is calculated by first determining the average  $\Delta C_T$  between mutant allele assays and reference assays run on multiple wild type samples, then the detection  $\Delta C_T$  cutoff value is derived by subtracting a value of 3 times the standard deviation or 2  $C_T$ s, whichever is larger. This is done to ensure that there is a significant difference between true amplification of a mutant allele and non-specific, off-target amplification. If a more conservative cutoff value is desired, 3  $C_T$ s can be subtracted from the average  $\Delta C_T$  value.

**6. Why is rounding high calculated detection  $\Delta C_T$  cutoff values to 9.96  $C_T$ s recommended?**

Many of the TaqMan® Mutation Detection Assays are capable of detecting very rare amounts of a mutant allele in comparison to a high background of wild type allele. Detection  $\Delta C_T$  values calculated for some sample types (e.g. cell line gDNA samples), can be larger than 9.96  $\Delta C_T$ s for many assays. Generally, we recommend that calculated  $\Delta C_T$  cutoff values that exceed 9.96  $\Delta C_T$ s are rounded down to 9.96, which is equivalent to 0.1% mutation detection. The Life Technologies predetermined detection  $\Delta C_T$  cutoff values for the validated assay set were rounded down to 9.96  $\Delta C_T$  where applicable, and the corresponding 0.1% sensitivity level was validated in experiments using samples containing synthetic mutant DNA template spiked into cell line and FFPE cell line gDNAs. For most applications, a lower sensitivity limit of 0.1% mutation detection is sufficient, however, one can use the actual calculated  $\Delta C_T$  cutoff value versus a rounded off value, if desired.

**7. What are the differences between the validated assay set and standard assays?**



The first set of TaqMan® Mutation Detection Assays underwent extensive validation. This set of validated assays covers 14 KRAS targets, 29 EGFR targets, and the BRAF V600E mutation targets and is referred to as the validated assay set. The main differences between the validated assays compared to other TaqMan® Mutation Detection Assays is that the validated assays include the following attributes:

- The mutant allele assays have corresponding wild type allele assays in addition to corresponding gene reference assays. These assays enable more accurate quantitation of the percent mutation in a sample.
- The calibration  $\Delta C_T$  values, which represent the inherent  $C_T$  difference between a mutant allele assay and its corresponding gene reference assay or wild type allele assay, were predetermined, enabling quantitative analysis of percent mutation in a sample
- Detection  $\Delta C_T$  cutoff values were predetermined for the validated assay set.

**Note:** The predetermined calibration  $\Delta C_T$  values and detection  $\Delta C_T$  cutoff values are provided in the TaqMan® Mutation Detection Assay Index file, which can be downloaded from: [www.lifetechnologies.com/castpcr](http://www.lifetechnologies.com/castpcr). They are also found in the 'TMDA\_assay\_attribute\_file.txt' file which is located in the Mutation Detector™ Software installation folder and in the software Assay Attributes table when these assays are present in a mutation analysis.

#### 8. Do the mutant allele assays cross react with other mutations?

Some mutant allele assays could potentially exhibit cross reactivity with non-target mutations that are found at the same or nearby genomic nucleotide positions as the target mutation. For example, an assay that detects a specific EGFR exon 19 mutation might also show some amplification signal with samples containing other, non-target, exon 19 mutations due to mismatch base pairing by the allele-specific primer. If a sample containing a non-target mutation gives a positive result, it is likely to be caused by cross reactivity.

For the validated TaqMan® Mutation Detection Assay set, experiments were conducted to investigate and establish cross reactivity patterns for mutant allele assays run with synthetic DNA templates containing mutations. No significant cross reactivity ( $\geq 0.1\%$ ) was detected for:

- The ten KRAS mutant allele assays that detect codon 12 and 13 mutations tested with other codon 12 and 13 mutations.
- The four KRAS mutant allele assays that detect codon 61 mutations tested with other codon 61 mutations.
- The three EGFR mutant allele assays that detect codon 719 mutations tested with other codon 719 mutations.
- The BRAF\_476\_mu assay to the V600E mutation tested with 6 other known BRAF codon 600 mutations; minor cross reactivity ( $<1\%$ ) was detected for 6 other mutations.
- Some of the individual assays to 19 deletions in EGFR exon 19 tested with other exon 19 deletions. Some individual exon 19 deletion assays exhibited higher levels of cross reactivity when run with deletions that shared significant sequence similarity to the target deletion.

Cross reactivity studies have not been run for other mutant allele assays, but judging from the studies done with the validated assay set, mutant allele assays to point mutations in codons are expected to have no significant cross reactivity with other point mutations in the same or nearby codons. Mutant allele assays designed to detect deletion mutations may have a low level of cross reactivity with deletion mutations that are similar in sequence. As well, assays that detect single point mutations may detect related multiple nucleotide mutations if the single point mutation is positioned at the first or last base of a multiple nucleotide mutation.

# PCR Good Laboratory Practices

PCR assays require special laboratory practices to avoid false-positive amplifications. The high-throughput and repetition of these assays can lead to amplification of one DNA molecule.

When preparing samples for PCR amplification:

- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Centrifuge tubes before opening. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNAZap<sup>™</sup> Solution (Part no. AM9890).





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## Chemical safety

### General chemical safety

#### Chemical hazard warning



**WARNING! CHEMICAL HAZARD.** Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.



**WARNING! CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

#### Chemical safety guidelines

To minimize the hazards of chemicals:

- 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. (See [“About SDSs” on page 46.](#))
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

## SDSs

### About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

### Obtaining SDSs

The SDS for any chemical supplied by Life Technologies is available to you free 24 hours a day. To obtain SDSs:

1. Go to [www.lifetechnologies.com](http://www.lifetechnologies.com), click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
  - **Open** – To view the document
  - **Print Target** – To print the document
  - **Save Target As** – To download a PDF version of the document to a destination that you choose

**Note:** For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

## Chemical waste safety

### Chemical waste hazards



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**CAUTION! HAZARDOUS WASTE.** Refer to Safety Data Sheets and local regulations for handling and disposal.

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**WARNING! CHEMICAL WASTE HAZARD.** Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

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**WARNING! CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

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## Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

## Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

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**IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

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Biological hazard  
safety

## General biohazard



**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. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* found at: [www.cdc.gov/biosafety/publications/index.htm](http://www.cdc.gov/biosafety/publications/index.htm)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; [www.access.gpo.gov/nara/cfr/waisidx\\_01/29cfr1910a\\_01.html](http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html)).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

[www.cdc.gov](http://www.cdc.gov)



# Documentation and Support

## Documentation

Portable document format (PDF) versions of the documents listed in this section are available from the Life Technologies website. See [“Obtaining support” on page 50](#).

**Note:** To open the PDF versions, use the Adobe® Acrobat® Reader software available from [www.adobe.com](http://www.adobe.com)

### Product documentation

Document	Part no.
<i>TaqMan® Mutation Detection Assays Protocol</i>	4467011
<i>TaqMan® Mutation Detection Assays Quick Reference Card</i>	4467012
<i>Mutation Detector™ Software Quick Reference Card</i>	4467104
<i>Mutation Detector™ Software User Guide</i>	4467102

### Instrument documentation

<i>Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Getting Started Guide for Standard Curve Experiments</i>	4387779
<i>Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Getting Started Guide for Comparative C<sub>T</sub>/Relative Standard Curve Experiments</i>	4387783
<i>Applied Biosystems 7900HT Fast Real-Time PCR System Absolute Quantification Getting Started Guide</i>	4364014
<i>Applied Biosystems StepOne® and StepOnePlus® Real-Time PCR Systems Relative Standard Curve and Comparative C<sub>T</sub> Experiments Getting Started Guide</i>	4376785
<i>Applied Biosystems StepOne® and StepOnePlus® Real-Time PCR Systems Getting Started Guide for Standard Curve Experiments</i>	4376784
<i>Applied Biosystems ViiA™ 7 Real-Time PCR System Getting Started Guides</i>	4441434
<i>Applied Biosystems ViiA™ 7 Real-Time PCR System User Guide</i>	4442661
<i>Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Getting Started Guides</i>	4470935
<i>Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System User Guide</i>	4470542

## Obtaining SDSs

Safety Data Sheets (SDSs) are available from [www.lifetechnologies.com/sds](http://www.lifetechnologies.com/sds)

**Note:** For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

## Obtaining support

For the latest services and support information for all locations, go to:

[www.lifetechnologies.com](http://www.lifetechnologies.com)

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

## 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 found on Life Technologies' website at [www.lifetechnologies.com/termsandconditions](http://www.lifetechnologies.com/termsandconditions). If you have any questions, please contact Life Technologies at [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

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**For support visit** [www.appliedbiosystems.com/support](http://www.appliedbiosystems.com/support)

[www.lifetechnologies.com](http://www.lifetechnologies.com)

