QUICK REFERENCE

Mutation Detector™ Software

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Contents

Purpose of the Mutation Detector™ Software

- Import instrument results files (*.csv or *.txt files) from TaqMan® Mutation Detection Assay experiments that were run and analyzed on supported Applied Biosystems® real-time PCR systems
- Perform mutation detection analyses to determine the:
  - Mutant allele assay detection ΔC_T cutoff values for your sample type
  - Presence or absence of a mutation
  - Percent mutation in samples when a calibration ΔC_T value is available

Well Data table

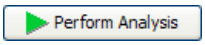
The Well Data table displays well-level data for all reaction plates included in the study.

Column	Description	Edits allowed?
Plate	The reaction plate name **Note:** If no reaction plate name is provided in the instrument results file, the *.csv or *.txt file name is used.	No
Well	The well number from the reaction plate	No
Assay	The name of the assay in the well	No
Sample	The name of the sample in the well	No
Control	(If applicable) The control assigned to the well: NTC, POSITIVE, or NEGATIVE If you change the control status of a well, you must reanalyze the data to account for the change. Click **Perform Analysis**. **Note:** IPCs are assigned according to the instrument results files exported from the real-time PCR system software. If you included IPC reagents in your PCR 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 reaction (FAM™ dye) and the IPC reaction (VIC® dye). IPCs cannot be edited in the Mutation Detector™ Software.	Yes
Sample Ct	The C_T value of the sample	No
IPC Ct	The C_T value of the IPC	No
Quantity	The numeric value amount of sample in the well (typically in reference to ng amounts or number of copies).	No
Omitted	The omit status of the well When the checkbox is selected, the well is omitted from the analysis. The Well Flag column contains a flag that explains why the well was omitted. If you change the omit status of a well, you must reanalyze the data to account for the change. Click **Perform Analysis**.	Yes
Well Flag	A flag that explains why the well was omitted from the analysis In the Well Data table, the software assigns only one flag per well. The flag hierarchy is: NOFAM, ACTEC, IPCEC, OFILE, CTOUT, OUSER.	No

Flags in the Well Data table

Flag	Description
NOFAM	The well contains VIC® dye C_T values, but no assay FAM™ dye C_T values are detected.
ACTEC	The FAM™ dye C_T value for the assay exceeds the cutoff value.
IPCEC	Both the VIC® dye C_T value for the Internal Positive Control and the FAM™ dye C_T value for the assay exceed the cutoff values.
OFILE	There was an omitted well in the instrument results file (*.csv or *.txt file).
CTOUT	The software omitted a C_T outlier in a replicate set.
OUSER	A user omitted the well in the Mutation Detector Software.

Assign or change a control

1. Click the **Well Data** tab.
2. For the well that you want to change, click inside the Control column cell.
3. Select **NTC**, **POSITIVE**, or **NEGATIVE** from the drop-down list. All related replicate wells are automatically populated with the same control name. The controls are defined in the table below.
4. Click 

to reanalyze the data.

Column	Description
NTC	No Template Control By default, samples that were named **NTC** in the instrument results files exported from the real-time PCR system software are labeled as NTCs in the Mutation Detector™ Software. However, you can change the NTC assignment in the Mutation Detector Software.
POSITIVE	A positive control is an assay run on a sample containing 100% on-target templates (i.e.,gene reference assays run on a wild type sample or mutant allele assays run on a sample that contains 100% mutant allele). There are two uses for positive controls: • Test for proper assay amplification of the assay target. If the positive control fails the C_T cutoff value, all wells with that assay are excluded from mutation detection calculations. • Real-time calculation of calibration ΔC_T values.
NEGATIVE	A negative control is a mutant allele assay run on a sample containing 100% wild type allele. Negative controls are used for real-time calculation of detection ΔC_T cutoff values. Note the following: • When a mutant allele assay negative control sample is selected, all wells containing other mutant allele assays run on the same sample are automatically selected as Negative controls by the software. • If a mutant allele assay negative control sample is selected, and a gene reference assay was run with the same sample, then the software automatically calculates a detection ΔC_T cutoff value for the mutant allele assay. If a calibration ΔC_T value for the assay pair is available, then this value will be used to calculate a normalized detection ΔC_T cutoff value for the mutant allele assay. • You cannot assign negative controls to wells that contain gene reference assays.

Replicates Average table

The Replicates Average table displays the sample-level data for all reaction plates included in the study. The table below describes each column in the Replicates Average table.

Note: You cannot edit the data in the Replicates Average table.

Note: Technical replicates are not required for mutation detection analysis, but we strongly recommend that you use technical replicates for real-time calculation of detection ΔC_T cut-off values.

Column	Description
Plate	The reaction plate name **Note:** If no reaction plate name is provided in the instrument results file, the *.csv or *.txt file name is used.
Assay	The name of the assay run with the sample
Sample	The sample name
Quantity	The numeric value amount of sample in the well (typically in reference to ng amounts or number of copies)
Control	The control assigned to the sample
#Replicates	The number of technical replicates The software determines the number of technical replicates by counting all wells on the same reaction plate that have the same sample name, assay name, and DNA quantity (if not provided, the DNA quantity is assumed to be equal).
#Valid Replicates	The number of valid technical replicates The software determines the number of valid technical replicates by counting only those wells that have not been omitted from the analysis. View the Well Data table Omitted and Well Flag columns to discern which replicate(s) have been omitted and why.
Avg Ct	The average C_T value for the valid technical replicates
Std Dev	Standard deviation of the valid technical replicates
Omitted	Samples that have been omitted from the analysis because all wells for the sample were omitted To include omitted wells in the analysis, you can: • Change the omit status in the Well Data table. • Edit the appropriate analysis settings in the Current Study Settings tab.
Flag	A flag that explains why the sample was omitted from the analysis **Note:** Multiple flags may appear for a sample if more than one flag applies.

Flags in the Replicates Average table

Flag	Description
NTCCT	The assay C_T value for the No Template Control is below the cutoff value (amplification was detected).
PCTEC	The assay FAM™ dye C_T value for the positive control sample exceeds the positive control C_T cutoff value.
RCTOR	The FAM™ dye C_T value for the reference assay is outside of the defined range.
NTYPE	The assay type is unknown. The assay name does not have the correct format or is not specified in the Assay Attributes file.

Assay Attributes table

The Assay Attributes table displays the *predetermined* and (if applicable) the *real-time calculated* calibration ΔC_T values and detection ΔC_T cutoff values that are used in the mutation detection calculations. The table below describes each column or button in the Assay Attributes table.

Column/button	Description	Edits allowed?
Mutant Assay	The name of the mutant allele assay	No
GeneRef Assay	The name of the gene reference assay that is paired with the mutant allele assay	No
Calibration ΔCt(-gr)	The predetermined calibration ΔC_T values for paired mutant allele assays and gene reference assays	Yes
Cal.Calibr. ΔCt(-gr)	The calculated calibration ΔC_T values for paired mutant allele assays and gene reference assays, when the required positive control data for the calculation exists in the current study	No

Column/button	Description	Edits allowed?
Detection ΔCt Cutoff	The predetermined detection ΔC_T cutoff values The cutoff value is the limit of detection that is used to determine the presence or absence of a mutant allele in a sample. For assays having calibration ΔC_T values and therefore normalized detection ΔC_T cutoff values, the detection ΔC_T cutoff values are converted to % in the Results table.	Yes
Cal. Detection ΔCt Cutoff	The calculated detection ΔC_T cutoff values, when the required negative control and gene reference assay data for the calculation exists in the current study. The cutoff value is the limit of detection that is used to determine the presence or absence of a mutant allele in a sample. If you select real-time calculated detection ΔC_T cutoff values, these values are shown in the results table. If a calibration ΔC_T value is available for the assay, then these values are converted to % in the Results table when the 'Show % Mutation' option is selected.	No
Reset button	Resets all edited values in the table to the predetermined values previously used in the current study	N/A
Import button	Imports a custom Assay Attributes file that contains user-defined predetermined values	N/A
Default button	Restores all edited values in the table to the default predetermined values from the 'TMDA_default_assay_attributes.txt' file stored in your Mutation Detector Software installation folder.	N/A

Results table

Each row in the Results table displays the analysis results for a sample run with a mutant allele assay paired with a wild type allele assay or a gene reference assay. The software compares the calculated normalized ΔC_T value to the detection ΔC_T cutoff value for the mutant allele assay. The table below describes each column in the Results table.

Note: You cannot edit the data in the Results table.

Column	Description
Plate	The reaction plate name **Note:** If no reaction plate name is provided in the instrument results file, the *.csv or *.txt file name is used.
Sample	The sample name
Quantity	The numeric value amount of sample in the well (typically in reference to ng amounts or number of copies)
Assay	The name of the mutant allele assay
Detection Cutoff	The detection cutoff value for the mutant allele assay, expressed as expressed as a ΔC_T value (from the Assay Attributes table) The detection cutoff value is expressed as a percentage for assays having calibration ΔC_T and normalized detection ΔC_T cutoff values, when the 'Show % Mutation' analysis option is selected. *% detection = $1/2^{\Delta C_T}$ × 100%* The software allows a minimum % detection cutoff value of 0.001%. The software does not limit the maximum detection C_T cutoff value used for a 'yes/no' mutation detected answer. **Note:** A detection ΔC_T cutoff value is required for mutation detection analysis. If no detection ΔC_T cutoff value is provided in the Assay Attributes table for the analysis, then the mutation detection calculation cannot be performed, and a NOCUT flag is displayed in the results table.

Column	Description
Detected?/%Mutation **Note:** Select/deselect the **Show % Mutation** button to toggle between the Detected? view and the % mutation view. The Show % Mutation option is available only for assays with calibration ΔC_T values.	**Detected?** – The calculated mutation detection results The results are displayed as follows: • Y = yes; the amount of mutation detected is below the mutation detection cutoff ΔC_T value and/or exceeds the percent mutation detection cutoff value for the mutant allele assay. That is, the sample ΔC_T value is less than the detection ΔC_T cutoff value. • N = no; the sample is either mutation negative, or below the limit of detection for the mutant allele assay. That is, the sample ΔC_T value is greater than the detection ΔC_T cutoff value. • I = invalid result; a flag or flags indicating the reason for the failure are displayed in the Flag column.
	% Mutation – The calculated amount of mutant allele within a mutation-positive sample for assays with calibration ΔC_T values. Quantitative analysis is not an option for mutant allele assay and gene reference assay pairs that have not been calibrated. For a sample in which a mutation was not detected, the software does not calculate a % mutation, but assigns 0%.
#Valid Replicates (mu)	The number of valid technical replicates for the mutant allele assay The software determines the number of valid technical replicates by counting only those wells that have not been omitted from the analysis. View the Well Data table Omitted and Well Flag columns to discern which replicate(s) have been omitted and why.
Avg Ct (mu)	The average C_T value of the valid technical replicates for the mutant allele assay
Std Dev (mu)	Standard deviation of the valid technical replicates for the mutant allele assay
Ref Assay	The name of the wild type allele assay or gene reference assay that is paired with the mutant allele assay for mutation detection analysis
#Valid Replicates (rf)	The number of valid technical replicates for the wild type allele assay or gene reference assay The software determines the number of valid technical replicates by counting only those wells that have not been omitted from the analysis. View the Well Data table Omitted and Well Flag columns to discern which replicate(s) have been omitted and why.
Avg Ct (rf)	The average C_T value of the valid technical replicates for the wild type allele assay or gene reference assay
Std Dev (rf)	Standard deviation of the valid technical replicates for the wild type allele assay or gene reference assay
ΔCt	The ΔC_T value of the mutant allele assay minus the wild type allele assay or gene reference assay in this sample
Calibration ΔCt	The calibration ΔC_T value of the paired mutant allele assay and wild type allele assay or gene reference assay (from the Assay Attributes table) that is used to calculate the normalized ΔC_T value
ΔCt_{norm}	Normalized ΔC_T value (the sample ΔC_T value minus the calibration ΔC_T) The normalized ΔC_T value is compared to the detection ΔC_T cutoff value for the mutant allele assay to determine sample mutation status.
Flag	Flags that explain why a valid mutation detection result could not be calculated for the sample **Note:** Multiple flags may appear for a sample if more than one flag applies.

Flags in the Results table

Flag	Description
MUPOS	A mutant allele assay run on a 100% mutant allele sample is selected as a positive control in the Well Data table. **Note:** The MUPOS flag appears when the corresponding assay samples are selected as positive controls in the Well Data table.
NOREF	There is no wild type allele assay or gene reference assay that pairs with the mutant allele assay for the same sample on the plate.
NOCUT	No Detection ΔC_T cutoff value (predetermined or real-time calculated) is available in the Assay Attributes table for the mutation analysis.

Flag	Description
Additional flags	After the software performs the analysis, all flags relevant to the mutation detection calculations are shown in the Results table, including: • Replicates Average table flags, except NTYPE (see page 3) • Well Data table flags, if all wells for a sample were omitted (see page 2)

Detection ΔC_T Cutoff Calculations table

The Detection ΔC_T Cutoff Calculations table is dynamically displayed when a detection ΔC_T cutoff value determination experiment is included in the Current Study. Each row in the ΔC_T Cutoff Calculations table displays all the data used to determine a ΔC_T cutoff value. In a typical experiment, three or more wild type gDNA samples are run with mutant allele assays (negative controls selected in the Well Data table) and paired gene reference assays. The software calculates a mutant allele assay ΔC_T cutoff value when a calibration ΔC_T value is not available and a normalized ΔC_T cutoff value when a calibration ΔC_T value is available. The table below describes each column in the ΔC_T Cutoff Calculations table.

Flag	Description
Assay	The name of the mutant allele assay.
Sample	The wild type sample name(s). Multiple values are separated by commas.
C_T(mu)	The C_T value(s) for the mutant allele assay (from the Replicates Average table). Multiple values are separated by commas. For any C_T values that are undetermined or greater than the Maximum C_T cutoff value (set in the Current Study Settings), the Maximum C_T cutoff value is shown and is used for the ΔC_T cutoff calculations.
Ref Assay	The name of the gene reference assay that is paired with the mutant allele assay for the detection ΔC_T cutoff determination analysis.
C_T(ref)	The C_T value(s) for the gene reference assay (from the Replicates Average table). Multiple values are separated by commas
ΔC_T	The ΔC_T value(s) of the mutant allele assay minus the gene reference assay in this sample. Multiple values are separated by commas
Ave ΔC_T	The average ΔC_T value for all wild type samples run with the same paired mutant allele assay and gene reference assay
Std Dev	Standard deviation of the sample ΔC_T values
ΔC_T Cutoff	ΔC_T = *[C_T(mutant allele assay negative control) – C_T(gene reference assay)]– (3 × the standard deviation or 2 C_T, whichever is greater)*
Calibration ΔC_T Cutoff (-gr)	The calibration ΔC_T value of the paired mutant allele assay and gene reference assay (from the Assay Attributes table), if available, that is used to calculate the normalized ΔC_T value
ΔC_T norm	*Normalized ΔC_T = [C_T(mutant allele assay negative control) – C_T(gene reference assay)] - Calibration ΔC_T - (3 × the standard deviation or 2 C_T, whichever is greater).*

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