Mutector™

Mutation Detection Kit

CALR

Cat No. GP20
32 reactions

User Manual V1.2

TrimGen
GENETIC DIAGNOSTICS

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CONTENTS

Storage

Upon receipt of the kit, store at –20ºC until use. At this temperature the reagents are stable for 6 months.

After first use, store all of reagents at 2-8ºC and keep them protected from direct light. At this condition the reagents are stable for 1 month.

Notice to Purchaser

The Mutector™ kit is provided for research use only, not for use in diagnostic procedures. The purchaser must determine the suitability of the product for their particular use.

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When working with the kit reagents, always wear a lab coat, disposable gloves, and protective goggles. TrimGen Corporation shall not be liable for any direct, indirect, consequential or incidental damages arising out of the misuse, the results of use, or the inability to use this product.

Trademarks

Introduction

Mutector™ CALR Mutation Analysis is designed to detect deletions and insertions in exon 9 of the Calreticulin gene.

Mutation	Nucleotide Change
L367fs*46	c.1092_1143del52
K385fs*47	c.1154_1155ins TTGTC
Others	any insertion/deletion between codons 360-386 of exon 9
L367fs*46 and K385fs*47 are two most common CALR mutations	

The mutation is detected by PCR and capillary electrophoresis. The PCR products are analyzed on an Applied Biosystems Genetic Analyzer using fragment analysis software. Each kit provides reagents enough for 32 reactions.

Materials Provided:

The Mutector™ CALR Mutation Analysis kit contains reagents enough for 32 tests.

Reagents	Quantity	Description
Master Mix	650 µl	Master Mix Reagents for DNA amplification
CALR DP*	45 µl	PCR primer mix for amplification of exon 9 of CALR gene
CALR CTL	45 µl	Mutation controls for CALR gene
Loading Buffer*	1000 µl	Sample loading buffer with size standards

* **Light Sensitive:** Keep these reagents protected from direct light.

Materials required:

0.2 ml PCR tubes (8-well strip tube)

DS-32 Matrix Standard kit (Applied Biosystems Cat. No. 4345831). This kit is a one-time calibration to set up the correct spectral channels. This is required for all Mutector II assays.

Equipment required:

Thermal Cycler:
Any type of thermal cycler with a 0.2 ml tube block is acceptable for performing the assay.

Sequencer:
Applied Biosystems Genetic Analyzer

Instrument	Data Collection	Data Analysis
Genetic analyzer 3100	Data Collection Software v3.0 or v3.1	GeneMapper® Software v4.0 or v4.1
Genetic analyzer 3700		
Genetic analyzer 3130		
Genetic analyzer 3500	3500 Data Collection Software v1.0	GeneMapper® Software v4.1

The Mutector products have been validated with the instruments listed above. However, this assay could apply to any capillary electrophoresis instrument that has fragment analysis feature. For instruments that are not listed above, customers should perform validation on their own instrument.

DNA Sample Preparation

The assay is compatible with DNA samples extracted using commercially available kit.
TrimGen provides DNA extraction kit for FFPE, FNA (fine needle aspiration) tissue samples.
The kit has been validated with this assay.

Product information:
WaxFree™ DNA for 50 samples (Cat. WF-50)
WaxFree™ DNA for 100 samples (Cat. WF-100)

To order: order@trimgen.com

DNA concentration:
When using a column or bead DNA extraction method, adjust the final concentration of extracted DNA to **20-80 ng /µl**
When using TrimGen's WaxFree DNA kit, follow the user manual to perform PCR reaction.

Sequencer Calibration

Spectral calibration is required before running the test

Spectral calibration is required for running STA assay, this is one time setup. Applied Biosystems DS-32 Matrix Standard (Cat No. 4345831) is used for calibration. Refer to the DS-32 kit manual to perform the spectral calibration. If your sequencer has already been calibrated by this Matrix Standard, then you can skip this step.

Setup Data Analysis Program

A one-time setup of the data analysis program is required for the first-time user of Mutector™ kit. After setup, the program can be applied for data analysis of all Mutector™ tests.

GeneMapper® Analysis

Step I. GeneMapper® Setup
www.trimgen.com/docs/PartI-GeneMapper-Setup.pdf

Step II. Data Collection® Software Setup
www.trimgen.com/docs/PartII-Data-Collection-Setup.pdf

Step III. Data Analysis Using GeneMapper®
www.trimgen.com/docs/PartIII-Data-Analysis-GeneMapper.pdf

Important

Spectral calibration is required before running the test

The sequencer needs to be calibrated with the DS-32 calibration kit (Applied Biosystems cat No. 4345831). This is a one-time calibration to set up spectral channels to collect the test results. Refer to the DS-32 Matrix standards kit to prepare the DS-32 matrix standards. Run a Matrix Standard Set DS-32 (5FAM, JOE, NED, ROX) to perform a spectral calibration.

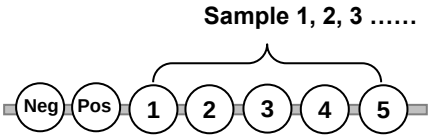
Assay Protocol:

A. PCR Amplification

Thaw all reagents and keep on ice. Spin down the reagents before use.

A negative control (water) is recommended to run with samples each time.

A.1. Collect 0.2 ml PCR strip tubes and label the tubes as follows:



Neg: Negative Control
Pos: CTL Control

A.2. Transfer **1 µl** of **CALR DP** into each tube.

A.3. Transfer **18 µl** of **Master Mix** into each tube.

A.4. Add **1 µl** of nuclease-free water to the "**Neg"** tube.

A.5. Add **1 µl** of **CALR CTL** to the **"Pos"** tube.

A.6. Add **1 µl*** of sample DNA (20-80 ng/µl) to each sample tube. When using TrimGen WaxFree kit for paraffin sample DNA extraction, add **1 µl*** final extract to each sample tube.

A.7. Place the PCR tubes in a thermal cycler and run **PCR Program.**

PCR Program	
1 cycle	94°C 5 min
35 cycles	94°C 30 sec 52°C 30 sec 72°C 30 sec
1 cycle	72°C 5 min
	Hold at 4°C

Optional: The PCR products can be verified by agarose gel electrophoresis (5 μl loading).

The procedure can be temporarily stopped after **PCR**.
The PCR products can be stored at 4°C for 1-2 days.

Sample Loading

B.1. Add **15 μl** of the **Loading buffer** to each well of a sequencer adapter plate.

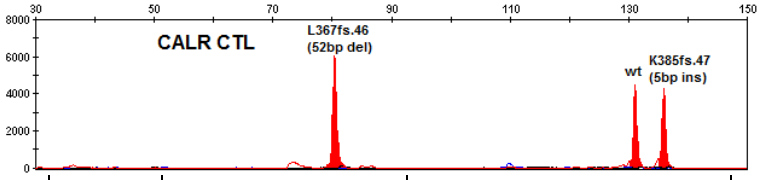
B.2. Transfer **2** to **5 μl** of the **PCR products** into each well and remove any bubbles in the well.

B.3. Load the plate to sequencer and run the pre-set Data Collection Program (ref. page 6).

C. Data Analysis

To analyze the results, zoom in on the area between 30 and 150 (see the figure below). The CALR CTL represents the wild type and two most common mutations: L367fs.46 (52-bp deletion) and K385fs.47 (5-bp insertion). Use these controls as standards to identify the peaks present in the test samples.

Results for CALR CTL

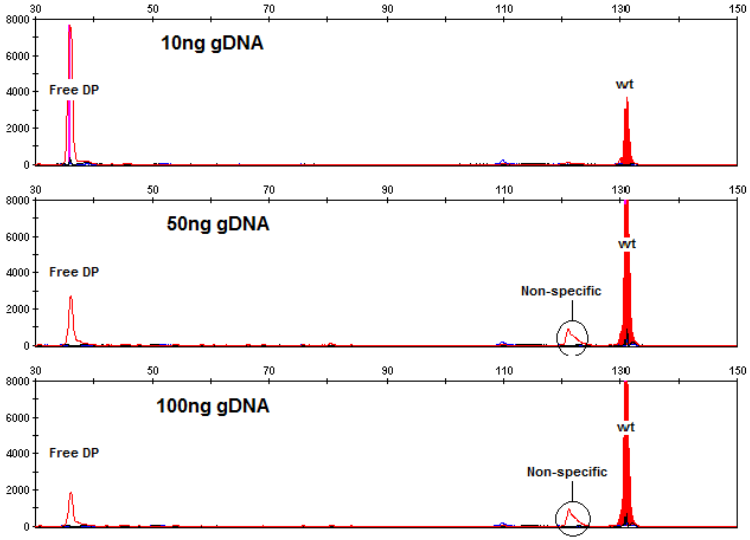


Peak #	Mutation	Nucleotide Change
1	L367fs.46	c.1092_1143del52
2	Wild Type	no change
3	K385fs.47	c.1154_1155insTTGTC

The wild type peak will be seen at approximately size 130 (may vary slightly by instrument and/or injection). All deletion mutation peaks are located between size 75 and the wild type peak (X-axis) and all insertion mutation peaks will appear between the wild peak and size 150 (X-axis).

Two small non-deletion peaks may be observed. One attached to the left of the wild type peak is a partial fragment of wild type (one base shortened); this small peak is not a deletion. The other small non-specific peak (see the circled peak in the DNA titration results below) may be observed approximately 10-bp shorter than the wild type peak, especially at higher input DNA amount. It should not be considered as a deletion peak.

Human genomic DNA titration results



The pattern, size or position of the peaks may vary slightly depending on instrument, polymer type and the length of capillary. **Customer should validate the correct size for each peak by using the CALR Control.**