Mutector™

Mutation Detection Kit

Warfarin

Genotyping Reagents (RUO)

User Manual V2.0

Cat No. GP03

www.trimgen.com

Limited Product Warranty

It is imperative that the users strictly adhere to this manual. Failure to do so will void TrimGen's guarantee of this product. TrimGen Corporation makes no other warranties of any kind, expressed or implied, including without limitation, warranties of merchantability or fitness for a particular purpose.

Notice to Purchaser

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

The purchase of the Mutector™ II kit includes a limited, nonexclusive license to use the kit. This license does not grant rights to reproduce or modify the Mutector™ II kit for resale, or to use the Mutector™ II kit to manufacture commercial products without written approval of TrimGen Corporation. No other license, expressed, implied or by estoppels is granted.

Product Safety and Liabilities

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.

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Storage

Upon receipt of the kit, store at –20ºC until use. At this temperature the reagents are stable for 8 months.

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

Introduction

The Mutector™ II Warfarin genotyping assay is a single tube test designed for identifying the following single nucleotide polymorphisms (SNPs):

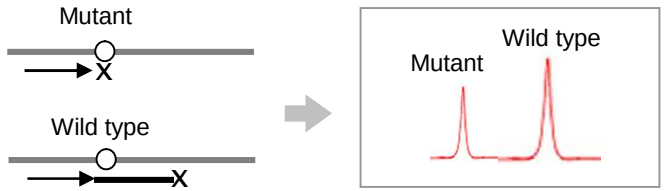
CYP2C9*2	C430T
CYP2C9*3	A1075C
VKORC1	-1639 G>A

The assay uses TrimGen's proprietary technology called Shifted Termination Assay (STA). The STA technology accurately detects single nucleotide variation through multiple steps: (1) Sequence-specific amplification of target gene (2) Sequence-selective termination of target nucleotide and (3) Sequence-dependent primer extension.

The genotypes are easily differentiated by fragment size and colors to give clear-cut results.

* Shifted Termination Assay (STA)

Shifted Termination Assay (STA) technology is a proprietary multi-base primer extension method. The specially formulated ST reagents extend detection primers by multiple bases to increase signal strength and fragment size, creating mutation peaks that are easily distinguished from wild type. The STA technology has sequencing like accuracy and the mutation is confirmed by both peak color and fragment size. The method enriches mutation signal and detects low-level mutations missed by sequencing.



STA reaction **Fragment analysis**

Overview of Warfarin Assay

Single tube assay for each sample

Step 1

PCR amplification
1-2 hours

Step 2

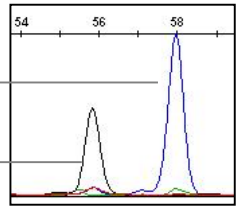
C-UP treatment
(PCR product clean-up)
30 min

Step 3

ST reaction (Genotyping)
45 min*

Load to sequencer

Step 4



Capillary Electrophoresis
Fragment analysis
30 min

Materials Provided:

The Mutector™ Warfarin Genotyping Kit contains reagents for 32 tests.

Materials	Cat. GP03	Description
Master Mix	650 µl	PCR Reagent Mix
PCR-P WR	70 µl	PCR Primers
C-UP 1	36 µl	PCR Product Clean UP Enzyme 1
C-UP 2	36 µl	PCR Product Clean UP Enzyme 2
C-UP Buffer	750 µl	Buffer for Clean UP
ST-WR*	770 µl	Reagent Mix for genotyping
DP- WR	70 µl	Detection Primers for genotyping
CTL- WR	20 µl	Control DNA for Alleles
Loading Buffer*	650 µl	Sample Loading Buffer with Size Standards

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

Reagents Description:

Master Mix
Reagents for DNA amplification.

PCR-P
PCR primer mix for amplification.

C-UP1 and C-UP2
Enzyme mix for cleanup of PCR products.

C-UP Buffer
Buffer for the C-UP enzymes.

ST-WR (Light sensitive)
Pre-mixed reagents for genotyping.

DP-WR
Pre-mixed primers for genotyping.

CTL-WR
Pre-mixed control DNA. The controls are sufficient for 10 test runs.

Loading Buffer (Light sensitive)
Loading buffer for ABI capillary type sequencers and special fluorescence-labeled size standards.

Materials required:

0.2 ml PCR tubes (8-well strip tube)

DS-32 Matrix Standard Kit for the 3100 and 3130 Series Systems (one time set up. Applied Biosystems Cat. No. 4345831)

Equipment required:

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

Sequencer:
Applied Biosystems capillary type Genetic/DNA Analyzer

Analysis Software:
Data Collection® software for ABI capillary sequencer
GeneMapper® for fragment analysis or GeneScan®

DNA Sample Preparation:

Any commercially available DNA extraction kit is acceptable.
DNA concentration:
When using a column or bead DNA extraction method, adjust the final concentration of extracted DNA to **50-200 ng/µL**.
When using TrimGen's DNA preparation kit, follow the kit protocol to perform the PCR reaction.

Sequencer setup:

First time users should set up the analysis program for the sequencer (one time setup). After setup, the program can apply to all Mutector™ II tests for data analysis. Please choose either GeneMapper® or GeneScan® to analyze your data.

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

GeneScan® Analysis

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

Step II. GeneScan® Setup and Data Analysis
www.trimgen.com/docs/PartIV-Genescan.pdf

Important

Spectral calibration is required before running the test

To read the test results correctly, 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 correct spectral channels to read 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.

Thermal Cycling Programs:

Program 1 (PCR)

1 cycle	94ºC 2 min
35 cycles	94ºC 20 sec 52ºC 30 sec 72ºC 30 sec
1 cycle	72ºC 1 min
	Hold at 4ºC

Program 2 (PCR product clean up)

	37ºC 25 min 95ºC 5 min
	Hold at 4ºC

Program 3 (ST reaction)

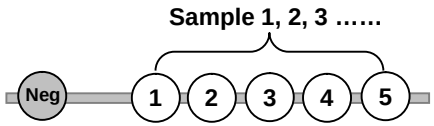
1 cycle	94ºC 1 minute
20 cycles	94ºC 20 sec 50ºC 45 sec 72ºC 10 sec
	Hold at 4ºC

Warfarin Assay Protocol:

A. PCR Amplification

Thaw all reagents and keep on ice. Spin down the reagents before use. A negative control (water) is suggested to run with the samples each time.

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



Neg: Negative control (water)

A.2. Prepare **PCR reaction mix**:

Master Mix = 18 x (_____ + 1*) x 1.1 = _____ µL**
Total number of sample

PCR Primers = 2 x (_____ + 1*) x 1.1 = _____ µL**
Total number of sample

*For negative and positive controls.

** For pipetting error.

Transfer entire volume of the reagents to one tube and mix the contents. This is the PCR reaction mix.

A.3. Add **20 µL** of PCR reaction mix into each tubes.

A.4. Add **2 µL** of nuclease-free water to the "**Neg**" tube.

A.5. Add **1-2 µL** of sample DNA (50-200 ng/µl) to each sample tube.

A.6. Place the PCR tubes in a thermal cycler and run **Program 1**.

Program 1	
1 cycle	94°C 2 min
35 cycles	94°C 20 sec 52°C 30 sec 72°C 30 sec
1 cycle	72°C 1 min
	Hold at 4°C

The procedure can be temporarily stopped after **Program 1**.
The PCR product can be stored at 4°C for next day test.

During the PCR amplification, prepare steps B1-B2.

B. PCR Product Clean-up

Prepare the C-UP mix

C-UP1 = 1 x number of samples x 1.1* =_____ µL

C-UP2 = 1x number of samples x 1.1* =_____ µL

C-UP Buffer = 9 x number of samples x 1.1* =_____ µL

* For pipetting error.

B.1. Collect 0.2ml strip tubes, one tube for each PCR reaction. Label the tubes the same as the PCR tubes.

B.2. Add **11 µL** of **C-UP Mix** to each new tube.

B.3. Transfer **5 µL** of PCR products to each corresponding tube.

B.4. Cap the tube, mix the contents and spin all tubes.

B.5. Incubate the tubes in a thermal cycler using **Program 2.**

Program 2
37°C for 25 min
95°C for 5 min
Hold at 4°C

During the incubation, prepare steps C1-C2.

C. STA Reaction (Genotyping)

C.1. Collect one 2 ml tube and label the tube with "**ST**". Prepare ST Mix using formula below:

ST Mix

ST-WR = 11 x (_____ + 1*) x 1.1 = _____µL**
Total number of sample

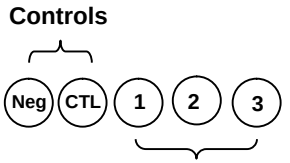
DP-WR = 2 x (_____ + 1*) x 1.1 = _____ µL**
Total number of sample

*For negative and positive controls.

** For pipetting error.

Transfer entire volume of the reagents to one tube and mix the contents. This is the ST mix.

C.2. Collect 0.2ml strip tubes, one for each PCR reaction. Label the tubes with sample ID. Label the tubes as follows:



C.3. Add **13 µL** of **ST Mix** (from step C.1) into each of the tubes.

C.4. Add **5 µL** of **CTL-WR** to the **"CTL"** tube.

C.5. Add **5 µL** of C-UP treated PCR products to each corresponding sample tube.

C.6. Mix the contents and spin all tubes.

C.7. Place the tubes into the thermal cycler and perform the ST reaction using **Program 3**.

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Program 3	
1 cycle	94°C 1 min
20 cycles	94°C 20 sec 50°C 45 sec 72°C 10 sec
	Hold at 4°C

D. Sample Loading

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

D.2. Transfer **5 µL** of the ST products into each well.

D.3. Load the plate to sequencer and run the pre-set Data Collection Program.

E. Data Analysis

User can set up the allele identification method using GeneMapper (detail see GeneMApper instruction). TrimGen also provide a third party analysis software, for detail information please contact us at "inforequest@trimgen.com".