Mutector™

Mutation Detection Kit

CYP 2C19

Genotyping Reagents

User Manual V1.3

Cat No. GP12

TrimGen
GENETIC DIAGNOSTICS

www.trimgen.com

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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 as 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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Trademarks

Introduction

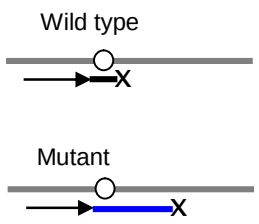
CYP2C19 Mutector™ Kit is comprised of two sets of reagents to detect and differentiate eight alleles: Set A reagents are used to detect alleles *2, *3, *4, and *5; Set B reagents are used to detect alleles *6, *7, *8, and *17.

CYP2C19 Alleles and Enzyme Activity

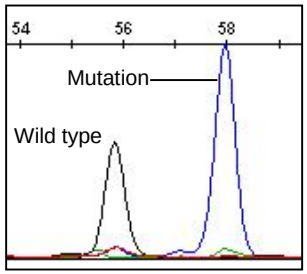
Allele	Genotype	Activity
*CYP2C19*2*	*c.681G>A*	None
*CYP2C19*3*	*c.636G>A*	None
*CYP2C19*4*	*c.1A>G*	None
*CYP2C19*5*	*c.1297C>T*	None
*CYP2C19*6*	*c.395G>A*	None
*CYP2C19*7*	*IVS5+2T>A*	None
*CYP2C19*8*	*c.358T>C*	Decreased
*CYP2C19*17*	*c.-806C>T*	Increased

* Shifted Termination Assay (STA)

Shifted Termination Assay is a proprietary technology that uses uniquely designed primers, mixtures of modified enzymes and specially synthesized nucleotides. STA technology extends primers by multiple bases to increase signal strength and fragment size, creating mutation peaks that are easily distinguished from wild type. The enriched mutation signals are then detected by fragment analysis.

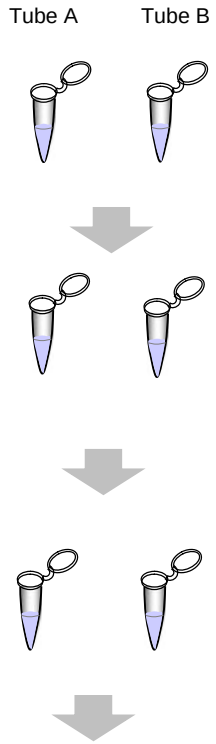


STA reaction



Fragment analysis

Overview of CYP2C19 Genotyping Assay



Tube -A for alleles *2, *3, *4, and *5

Tube-B for alleles *6, *7, *8, and *18

Materials Provided:

The Mutector™ CYP2C19 Genotyping contains reagents for 32 tests.

Materials	GP12	Description
Master Mix	2x 600 µl	PCR Reagent Mix
C-UP 1	65 µl	PCR Product Clean UP Enzyme 1
C-UP 2	65 µl	PCR Product Clean UP Enzyme 2
C-UP Buffer	780 µl	Buffer for Clean UP Enzymes
ST-F*	780 µl	Reagent Mix for Allele Detection
Loading Buffer*	2x 650 µl	Sample Loading Buffer with Size Standards
PCR-P A	70 µl	PCR Primers for *2, *3, *4, and *5
PCR-P B	70 µl	PCR Primers for *6, *7, *8, and *17
DP- A	70 µl	Detection Primers for *2, *3, *4, and *5
DP- B	70 µl	Detection Primers *6, *7, *8, and *17
CTL- A	50 µl	Control DNA for *2, *3, *4, and *5
CTL- B	50 µl	Control DNA for *6, *7, *8, and *17

* **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 for calibration of sequencer
(Applied Biosystems Pat No. 4345831)

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

DNA Sample Preparation:

Reagents for DNA preparation are not provided with the kit.
Any commercially available DNA extraction kit is acceptable.

DNA concentration adjustment:
When using a column or bead DNA extraction method, adjust the final concentration of extracted DNA to **100 ng/«l**.

Sequencer Setup:

First time users should set up the analysis program for the ABI sequencer (one time setup). After setup, the program can apply to all Mutector™ tests for data 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.

Thermal Cycling Programs:

Program 1 (PCR)

1 cycle	94ºC 2 min
35 cycles	94ºC 20 sec 53ºC 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 (STA Reaction)

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

Mutector™ Assay Protocol:

A. PCR Amplification

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

Negative and positive controls are recommended for each run.

§§ Tube A (alleles *2, *3, *4, and *5)

A.1. Prepare PCR Reaction Mix A:

Calculate the amount of Master Mix and PCR primers using following formula.

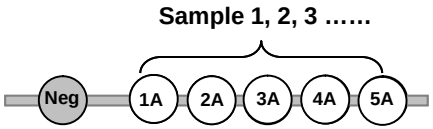
Master Mix = 18 µl x (_____) x 1.1* =_____ «l
# of Samples/Controls

PCR-P A = 2 µl x (_____) x 1.1 = _____ «l**
# of Samples/Controls

* Adjustment for pipetting error.

Label a tube with "**A**" and transfer the above reagents to the **"A"** tube and gently mix the contents (avoid bubble). This is the PCR Reaction Mix A.

A.2. Collect 0.2 ml PCR tubes, 1 tube per sample and label the tubes as shown below:



Neg: Negative control

A.3. Transfer **20** «**l** from the tube **"A"** into each of the tubes.

A.4. Add **2** «**l** of nuclease free water to the "**Neg"** tube.

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

§§ Tube-B (Alleles *6, *7, *8, and *17)

A.6. Prepare PCR Reaction Mix B:

Calculate the amount of Master Mix and PCR primers using following formula.

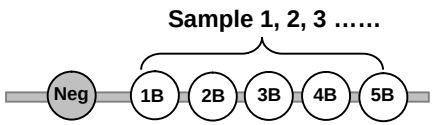
Master Mix = 18 µl x (_____) x 1.1* =_____ «l
# of Samples/Controls

PCR-P B = 2 µl x (_____) x 1.1 = _____ «l**
# of Samples/Controls

* Adjustment for pipetting error.

Label a tube with "**B**" and transfer the above reagents to the **"B"** tube, gently mix the contents (avoid bubble). This is the PCR Reaction Mix B.

A.7. Collect 0.2 ml PCR tubes, 1 tube per sample and label the tubes as shown below:



Neg: Negative control

A.8. Transfer **20** «**l** each from the tube "**B**" into all of the tubes.

A.9. Add **2** «**l** of nuclease free water to the "**Neg"** tube.

A.10. Add **1-2** «**l** of sample DNA (50-200 ng/µl) to each sample tube.

A.11. Cap the tubes and mix the contents. After spin, 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 53°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 products can be stored at 2-8°C for next day test.

B. PCR Products Clean Up

B.1. Prepare PCR Clean-Up (C-UP) Mix:

Calculate the amount of C-UP Buffer, C-UP1 and C-UP2 using following formula:

C-UP Buffer = 9 µl x (_____) x 1.2* = _____ µl
# of PCR tubes

C-UP1 = 1 µl x (_____) = _____ µl
# of PCR tubes

C-UP2 = 1 µl x (_____) = _____ µl
# of PCR samples

* Adjustment for pipetting error.

Label a tube with "C-UP" and transfer the above reagents to the **C-UP** tube and gently mix the contents (avoid bubble). This is the PCR Clean-Up Mix.

B.2. Collect 0.2 ml strip tubes, one tube for each PCR reaction. Label the tubes the same as the PCR tubes.

B.3. Transfer 11 **µl C-Up mix to new tube,**

B.4. After PCR reaction finished, spin the PCR tube and transfer 5 µl of **PCR product** to each C-Up tube (the remaining PCR products can be stored at –20°C for re-test).

B.5. Gently mix the content and spin the tubes.

B.6. Incubate the tubes in a thermal cycler using **Program 2**.

Program 2
37ºC for 25 min
95ºC for 5 min
Hold at 4ºC

The procedure can be temporarily stopped after **Program 2**. The C-UP products can be stored at 2-8ºC for next day test.

C. STA Reaction (Genotyping)

§§ Tube Set-A (Alleles *2, *3, *4, and *5)

C.1. Prepare STA Mix A (SA):

Label a tube as "**SA**".

Prepare "**SA**" mix as following:

ST-F Mix = 11 µl x (_____ + 1*) x 1.1 = _____µl**
# of C-UP tubes

DP-A = 2 µl x (_____ + 1*) x 1.1 = _____ µl**
# of C-UP tubes

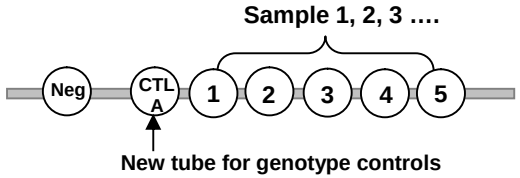
* For mutation controls

** Adjustment for pipetting error

Transfer the above reagents to the "**SA**" tube and gently mix the contents (avoid bubble). This is the STA Mix A.

C.2. Collect 0.2 ml strip tubes, one tube for each C-UP treated sample. **Add an extra new tube for genotype controls and label the tube with CTL.**

Tube Set-A



The CT- A is genotype controls designed for data analysis and should be run each time.

C.3. Add **13 µL** of "**SA"** into each tube of Tube Set-A.

C.4. Add **5 µL** of **2C19 CTL-A** into the **CTL-A tube**

C.5. Transfer **5 µL** of **C-UP treated PCR products** from the **Tube A** in C-UP section to each Set-A tube correspondingly.

C.6. Cap the tubes, mix the contents and spin the tubes.

§§ Tube Set-B (Alleles *6, *7, *8, and *17)

C.7. Prepare STA Mix B (SB):

Label a tube as "**SB**".

Prepare "**SB**" mix as following:

ST-F Mix = 11 µl x (_____ + 1*) x 1.1 = _____µl**
# of C-UP tubes

DP-B = 2 µl x (_____ + 1*) x 1.1 = _____ µl**
# of C-UP tubes

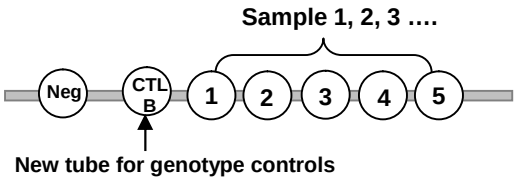
* For mutation controls

** Adjustment for pipetting error

Transfer the above reagents to the **"SB"** tube and gently mix the contents (avoid bubble). This is the STA Mix B.

C.8. Collect 0.2 ml strip tubes, one tube for each C-UP treated sample. **Add an extra new tube for genotype controls and label the tube with CTL.**

Tube Set-B



The CTL-B is genotype controls designed for data analysis and should be run each time.

C.9. Add **13 µL** of **"SB"** into each tube of Tube Set-B.

C.10. Add **5 µL** of **2C19 CTL-B** into the **CTL-B tube**

C.11. Transfer **5 µL** of **C-UP treated PCR products** from the **Tube B** in C-UP section to each Set-B tube correspondingly.

C.12. Cap the tubes, mix the contents and spin the tubes.

C.13. Place the tubes into the thermal cycler and perform the STA reaction using **Program 3.**

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

The procedure can be temporarily stopped after **Program 3**. The STA products can be stored at 2-8°C overnight.

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 STA reaction products into each well. Confirm and remove any bubbles in the well.

D.3. Load the plate into the sequencer and run the pre-setup 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".