**PG1502 DNA Detection Kit** CE 0120

REF **PG-1502C-024,** REF **PG-1502C-048,** REF **PG-1502C-096**

IVD **For *In Vitro* Diagnostic use**

## 1. Intended Use

The intended use for PG1502 DNA detection kit is to detect the presence of HLA-B*1502 allele in the blood samples of genetically at-risk populations.

## 2. Materials

### 2.1 Cont. Kit contents

| Vial | Reagent | Content and functions | Package size (Volume) | | |
|---|---|---|---|---|---|
| | | | 96 Rxn | 48 Rxn | 24 Rxn |
| Red | Genotype Detection Mix (10X conc.) | ◎ 10x conc. reagent containing the mix of forward and reverse primers for HLA-B*1502 allele.<br>◎ Use to amplify the specific region of human HLA-B*1502 allele. | 120 µl | 60 µl | 30 µl |
| Green | Internal Control Detection Mix (10x conc.) | ◎ 10x conc. reagent containing the mix of forward and reverse primers for internal control gene.<br>◎ Use to amplify the specific region of internal control gene. | 120 µl | 60 µl | 30 µl |
| Blue | Positive Control Template | ◎ Include the fragment of "HLA-B*1502 allele" and "Internal control gene" | 64 µl | 32 µl | 16 µl |
| Yellow | PCR Master Mix (2x conc.) | ◎ 2x conc. reagent containing SYBR GREEN I dye, Hot-start DNA polymerase, dNTPs, ROX reference dye and buffer。 | 1.2ml | 0.6ml | 300µl |
| Clear | Nuclease-Free Water | ◎ Use to adjust the final PCR reaction volume<br>◎ used as "No template Control".(NTC) | 1.2ml | 0.6ml | 300µl |

### 2.2 Material required but not provided

- ❖10 µl micro-pipette
- ❖20 µl micro-pipette
- ❖200 µl micro-pipette
- ❖1.5 ml tube with cap
- ❖96 well PCR optical plate
- ❖Optical adhesive film

### 2.3 Instrument required but not provided

- ❖Real-time PCR detection system selected from ABI 7500/7000, Bio-rad iQ5, Cepheid SmartCycler, Corbett Rotor-Gene 3000 or Roche LC480
- ❖DNA extraction system (Qiagen QIAamp DNA Mini Kits is recommended)

## 3. Introduction

### 3.1 Background:

HLA-B*1502 allele is found to have a strong linkage with Carbamazepine induced Steven-Johnson Syndrome (SJS) and Toxic Epidermal necrolysis (TEN) in Asian descendents. HLA-B*1502 allele is located in human chromosome 6 and belongs to human leukocyte antigen. The results of PG-1502 DNA detection can be used as an aid for the clinicians to identify the patients with the presence of HLA-B*1502 allele that may pose a greater risk of carbamazepine (CBZ) induced SJS/TEN.

### 3.2 Component Description:

PG1502 DNA detection kit contains HLA-B*1502 allele specific primers, controls, internal control mix, PCR master mix and nuclease-free water..

### 3.3 Number of Test:

Package with size of 96 reactions can be used to conduct a maximum of 46 specimens, size with 48 reactions for a maximum of 22 specimens, and size with 24 reactions for maximum 10 specimens. The final volume of each reaction is 25µl.

### 3.4 Storage/ Stability:

The kits should be stored at-15~-20℃ and the unopened kit can be kept stable until the expired date. The expired date is 6 months after manufactured date. Stored at + 4℃ is not recommended. Do not subject the kit more than 4 freeze-thaw cycles. If the kits are not often to be used, aliquot and freeze the reagent is recommended.

### 3.5 Analytical performance:

516 blood samples were tested to obtain the performance of PG1502 DNA detection kit. The positive percent agreement of PG1502 DNA detection kit is greater than 99% and the negative percent agreement is 92.39%. The overall percent agreement between PG1502 DNA detection kit and sequence based typing is 93.41%.

### 3.6 Sample collection and handling:

Technician should wear safety equipments while handling the samples. The whole peripheral blood should be collected in a tube containing either sodium citrate or K2- EDTA as the anticoagulants. **Do Not Use the Heparin as the anticoagulant**. **Do Not Use hemolyzed blood sample**. It is highly recommended that DNA extraction should be performed either at the day of blood sample collection when stored in 4ºC. The assay should be performed as early as possible after DNA extraction, and no later than 7days of storage at 4ºC.

### 3.7 Interference:

According to laboratory data, the performance was not affected for the blood sample containing bilirubin less than 8mg/dL, lipid less than 150mg/dL or Aspirin less than 35µg/mL. However, decrease performance was observed with DNA samples containing 0.025% of hemoglobin and very high residual Qiagen wash buffer.

## 4. Protocol

### 4.1 HLA-B*1502 detection and Internal control detection (IC)

Two independent PCR reactions should be conducted simultaneously for each DNA sample. One reaction is for HLA-B*1502 allele detection (add Genotype Detection Mix of red-cap vial) and the other is for internal control gene detection (add Internal Control Detection Mix of green-cap vial). The purpose of internal control gene detection is to identify the false negative result caused by PCR inhibition.

4.1.1 **No template control (NTC)**

No template control should be tested in each run. When testing, use the supplied nuclease-free water (clear-cap vial) to replace the sample DNA.

4.1.2 **Positive control (PC)**

Positive control should be tested in each run. When testing, use the supplied positive control template to replace sample DNA.

### 4.2 Sample DNA

Add 2µl of sample DNA per reaction (DNA amount: should be within 25-100 ng/reaction). The sample DNA will be extracted from human whole blood of the patient, using the recommended DNA extraction system (See 2.3) The sample DNA OD260/280 ratio should be adjusted to between 1.7 and 2.0 using spectrophotometer..

### 4.3 Procedure

4.3.1 Defrost the reagent on ice. Gently mix. Place the reagent on ice after a quick centrifuge. **(Do Not Mix Different Lots in the Same Run)**

4.3.2 Set real-time PCR system amplification program and add dissociation curve stage as below:

| | Time | Temp. | Cycle | Description |
|---|---|---|---|---|
| 1. DNA polymerase hot-start step | 10 min | 95℃ | 1 | Hot-start DNA polymerase is activated by this step. |
| 2. Amplification cycle: | | | 35 | |
| Denaturation | 15 sec | 95℃ | | |
| Annealing/Extension | 1 min | 71℃ | | Fluorescence signal is collected in this step in each cycle. |

***add dissociation curve stage**

4.3.3 Master Mix Preparation. (The preparation process should be kept under 4℃)

| Step | Action |
|---|---|
| 1 | Solution A & B: Based on the number of reactions, calculated the required amount of the three components shown in the table. Then pipette the following components into a 1.5ml Vial. |
| 2 | ◎ Mix by vortex and spin-down briefly。<br>◎ Add 23 µl solution A into the red area and solution B into green area (See 7.1 Sample loading chart)<br>◎ Add 2 µl of sample DNA into two wells, one in red area, and the other in green area. Apply similar procedures to PC and NTC. For PC, add 2 µl positive control template (blue-cap vial). For NTC, add 2µl nuclease-free water (clear-cap vial). |
| 3 | Seal the PCR plate with an optical adhesive film. Quick centrifuge at 1500 rpm for 10 sec. |
| 4 | Place the PCR plate in real-time PCR instrument. |
| 5 | Instrument setup is described in 4.3.2 |

Step 1 components:

| Cap | Component | Solution A | Solution B | conc. |
|---|---|---|---|---|
| Yellow | 2x PCR Master Mix | 12.5µl | 12.5µl | 1x |
| Red | 10x Genotype Detection Mix | 2.5µl | - | 1x |
| Green | 10x Internal Control detection Mix | - | 2.5µl | |
| Clear | Nuclease-free water | 8µl | 8µl | |
| | Total volume | 23µl | 23µl | |

### 4.4 Data Analysis

After the PCR reaction, the value of threshold should be set manually for the PCR machine you use. The threshold value on the instrumental system is listed below

| Model of Real-Time PCR system | Threshold |
|---|---|
| ABI 7500(Fast) Real-time PCR System | 0.15 |
| ABI 7000 Sequence Detection System | 0.15 |
| Bio-Rad iCycler iQ5 Real-Time PCR Detection System | 150 |
| Cepheid SmartCycler System | 8.3 |
| Corbett Research Rotor-Gene 3000 | 0.1 |

Two Ct values for each sample will be obtained, one from Genotype Detection Mix and the other from Internal Control Detection Mix. The differences of Ct values are calculated according to the equation shown below.

$$\Delta Ct = Ct_{\text{Genotype Detection mix}} - Ct_{\text{Internal control detection mix}}$$

When the Ct value of internal control is equal to or less than 27, and the ΔCt value is equal to or less than 7, the result should be identified as "HLA-B*1502 allele positive". Whereas, the ΔCt value is greater than 7, the result should be identified as "HLA-B*1502 allele negative". When the Ct value of internal control is greater than 27, the PCR inhibition should be suspected and repeating the test is highly recommended.

4.4.1 Result identification chart

<table>
<tr><td rowspan="3">IC Ct ≤ 27</td><td rowspan="2">$Ct_{\text{Genotype Detection Mix}}$ ≤ 35</td><td>ΔCt ≤ 7</td><td>HLA-B*1502 positive</td></tr>
<tr><td>ΔCt > 7</td><td>HLA-B*1502 negative</td></tr>
<tr><td colspan="2">$Ct_{\text{Genotype Detection Mix}}$ > 35 (undetermined)</td><td>HLA-B*1502 negative</td></tr>
<tr><td rowspan="2">IC Ct > 27</td><td colspan="2">PCR inhibitors may be present in specimen</td><td rowspan="2">Retest</td></tr>
<tr><td colspan="2">Inappropriate gDNA quantity</td></tr>
</table>

( IC: internal control)

4.4.2 The results of PC and NTC should be confirmed to be acting normal before the sample data is processed. For PC, the Ct value of internal control should be equal to or less than 27 and ΔCt value should be equal to or less than 7. For no template control, the result should be undetermined or Ct>35.

4.4.3 If there is any question about data interpretations, please call PharmiGene service line: +886-02-2695-9800。

## 5. Warning and Caution ⚠ ☣

*◎ Technician should wear safety equipments。*

*◎ Warning: All products contain blood derivations should be treated as potentially infectious. No known test or method can confirm that human blood derivations will not transmit infectious material.*

*◎ These product components may cause irritant. Avoid contacting with eyes, skin or cloth.*

*◎ If contacting with eyes, skin or mucous, wash immediately with water.*

*◎ After experiment, completely clean the operation area and flush the splash area with plenty water.*

*◎ If the test is interrupted, repeat the test to collect the correct result.*

*◎ The Result can only be used as genotype identification. The use of Carbamazepine should be judged by clinical professionals.*

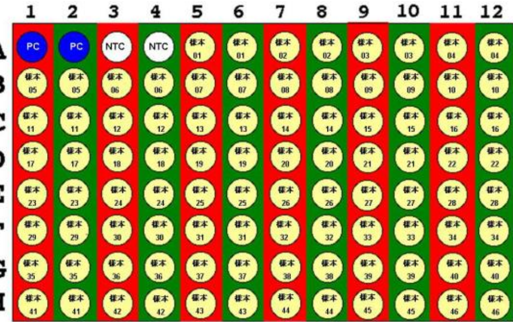
## 6. References

1) *Nature*, Vol. 428 , p.486 (2004)

2) *Pharmacogenetics and Genomics*, Vol. 16, p.297-306 (2006)

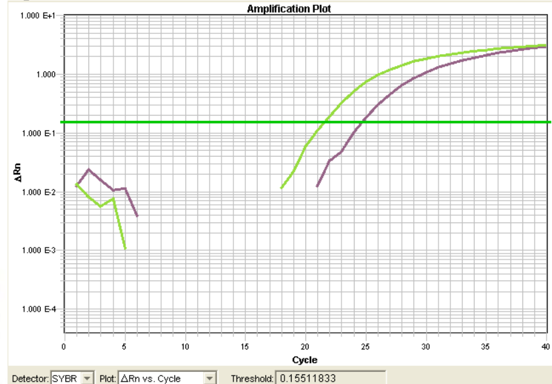
## 7. Appendix

Sample loading chart

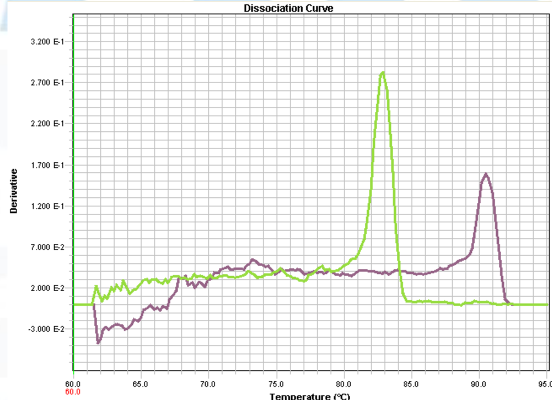



**PC: positive control NTC: no template control**
**Red area: for HLA-B*1502 allele detection**
**Green area: for internal control gene detection**

Amplification Plot




Dissociation Plot

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