

Part Numbers

PG-5801F-012 (12 reactions)
PG-5801F-024 (24 reactions)
PG-5801F-048 (48 reactions)
PG-5801F-096 (96 reactions)

1 Intended Use

The PG5801 FastGel Kit is intended for use in the detection of the HLA-B*5801 allele in the blood of individuals in genetically at-risk populations.

2 Materials

2.1 Kit Contents

Vial	Reagent	Contents	Kit size (Volume)			
			96 Rxn	48 Rxn	24 Rxn	12 Rxn
Red	10X Genotype Detection Mix	Concentrated mix of forward and reverse primers for amplification of the HLA-B*5801 allele and the internal control gene.	240 µl	120 µl	60 µl	30 µl
Blue	Positive Control Template	Includes target fragments of the HLA-B*5801 allele and the internal control gene.	64 µl	32 µl	16 µl	16 µl
Yellow	2X PCR Master Mix	Concentrated reagent containing Hot-Start DNA polymerase, dNTPs, and buffer.	1.2 ml	0.6 ml	0.3 ml	0.15 ml
Clear	Nuclease-Free Water	Water for final dilution of PCR reaction mixtures and for use as the No Template Control (NTC).	1.2 ml	0.6 ml	0.3 ml	0.15 ml

2.2 Materials required (not provided)

10 µl micro-pipette	1.5 mL tube with cap
20 µl micro-pipette	96-well PCR plate or PCR vial
200 µl micro-pipette	Adhesive film or cap

2.3 Instrumentation required (not provided)

- A. PCR thermocycler system
- B. DNA extraction system (QIAGEN® QIAamp® DNA Blood Mini Kit is recommended)

3 Introduction

3.1 Background

The HLA-B*5801 allele is strongly associated with Allopurinol induced severe cutaneous adverse reactions (SCARs) which include Hypersensitivity syndrome (HSS), Steven-Johnson Syndrome (SJS) and Toxic Epidermal necrolysis (TEN). HLA-B*5801 is located on chromosome 6 and belongs to the family of human leukocyte antigens. Detection of this allele can be used to identify patients who may be at greater risk of Allopurinol-induced SCAR.

3.2 Components

The PG5801 FastGel Kit contains primers specific to the HLA-B*5801 allele and an internal control gene, as well as positive control template, PCR master mix and nuclease-free water.

3.3 Number of tests per kit

The 96-reaction kit (PG-5801F-096) contains sufficient reagent to test 80 samples. The 48-reaction kit (PG-5801F-048) contains sufficient reagent to test 40 samples. The 24-reaction kit (PG-5801F-024) contains sufficient reagent to test 20 samples. The 12-reaction kit (PG-5801F-012) contains sufficient reagent to test 10 samples.

3.4 Storage and Stability

Kits should be stored frozen at -15~-20°C; unopened kits will remain stable until the expiration date. Storage at +4°C is not recommended. Do not subject reagents to more than 3 freeze-thaw cycles. If the kits are not to be used often, aliquot and freeze the reagents in smaller volumes.

3.5 Analytical Performance

The performance characteristics of the PG5801 FastGel Kit were determined from testing 100

blood samples. The sensitivity and specificity of PG5801 FastGel Kit are greater than 99%. PG5801 FastGel Kit is unable to distinguish the following rare allele types: HLA-B*5705, 5804, 5805, 5809, 5810, 5811, 5812, 5813, 5815, 5817, 5819, 5821, 5822, 5823, 5824 and 5828.

3.6 Sample Collection and Handling

Universal safety precautions should always be used when handling human samples. Whole peripheral blood should be collected in a tube containing either sodium citrate or K2-EDTA anticoagulants. **Do not use heparin as the anticoagulant. Do not use hemolyzed blood samples.** It is strongly recommended that DNA extraction be performed the day of sample collection. If this is not possible, the blood sample should be stored at +4°C; DNA extraction should occur as soon as possible after sample collection, and no later than 3 days of storage at +4°C.

3.7 Interference

Kit performance is not affected by the following interferents in blood:
<8 mg/dL bilirubin
<150 mg/dL lipid
<35 µg/mL salicylic acid (aspirin)
Decreased kit performance was observed with DNA samples containing 0.025% hemoglobin or high residual QIAGEN® wash buffer.

4 Protocol

4.1 HLA-B*5801 and Internal Control detection (IC)

One PCR reaction is conducted on each DNA sample to detect the HLA-B*5801 and the internal control gene. The internal control serves to identify possible false negative results by revealing the presence of interferents in the reaction mixture.

4.1.1 No Template Control (NTC)

A no template control should be tested in each run. Use 2 µl of the nuclease-free water (clear-cap vial) provided with the kit.

4.1.2 Positive Control (PC)

A positive control should be tested in each run. Use 2 µl of the positive control template (blue-cap vial) provided with the kit.

4.2 Sample DNA

Sample DNA is extracted from whole blood, preferably with the recommended DNA extraction system (See 2.3). The OD260/280 of the sample should be measured spectrophotometrically, and should be between 1.7 and 2.0. Use 2 µl of sample DNA per reaction (dilute sample DNA as needed to obtain 25-100 ng/reaction).

4.3 Procedure

4.3.1 Thaw the reagents on ice. Gently mix the reagents, and spin them briefly in a centrifuge before returning them to the ice. **Do not mix reagent lots.**

4.3.2 Program the PCR thermocycler system with PCR amplification stages as follows:

Hot-Start	10 min. @ 95°C	1 cycle	DNA polymerase activation
Denaturation	15 sec. @ 95°C	35 cycles	
Annealing/Extension	40 sec. @ 71°C		

4.3.3 Prepare the Master Mix. **Prepare your master mixes and reactions on ice.**

4.3.3.1 Prepare sufficient master mix volumes to accommodate n + 3 samples, with n = number of specimens to be tested. Add 3 to account for the positive and negative controls, and extra volume to accommodate pipetting error.

4.3.3.2 Prepare reaction mix as follows:

Cap	Reagent	Volume
Yellow	2X PCR Master Mix	12.5 µl
Red	10X Genotype Detection Mix	2.5 µl
Clear	Nuclease-free water	8 µl
Total Volume		23 µl

- 4.3.3.3 Briefly vortex and spin the reaction mixes, then aliquot 23 µl Mix into the well/vial.
- 4.3.3.4 Add 2 µl of sample DNA into well/vial. 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).
- 4.3.3.5 Seal the PCR well/vial with adhesive film or cap. Centrifuge briefly at 1500 rpm for 10 sec.
- 4.3.3.6 Place the plate in the PCR thermocycler system, and run the HLA-B*5801 program described in 4.3.2.

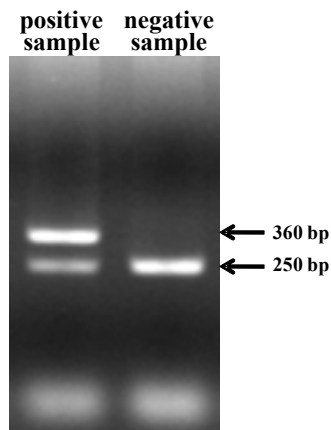
4.4 Data Analysis

Prepare 2% agarose gel for result identification. After the PCR reaction is completed, mix 10µl of amplified sample with 2µl 6x loading dye and load the mix (total 12µl) into the gel. Run the gel electrophoresis under 100 volts for 15~20 minutes at room temperature in TAE buffer.

4.4.1 Results Interpretation: the positive samples will have two PCR products with size of 360 bp and 250 bp when stain by EtBr. The negative samples will have only one PCR product with size of 250 bp.

Sample DNA type	Number of PCR products	Size of PCR products
HLA-B*5801 positive	2	360 and 250 bp
HLA-B*5801 negative	1	250 bp
NTC	0	-

4.4.2 Result output is as below:



4.4.3 For questions and assistance with the assay or with results interpretation, please call the Pharmigene service line at +866-2-2695-9800.

5. WARNINGS and PRECAUTIONS

**** Always use Universal Precautions when working with samples and reagents.**

**** All products containing blood derivatives should be treated as infectious. No known test or method can confirm that human blood derivatives will not transmit infectious material.**

**** Kit components may cause irritation. Avoid contact with eyes, skin, or clothing.**

**** If contact with the eyes, skin, or mucous membranes occurs, wash immediately with water.**

**** After testing is completed, completely clean the work area, taking care to cleanse splash areas, as well.**

**** If the test is interrupted, repeat the test from the beginning to ensure the collection of accurate data.**

**** Results may only be used for genotype identification. The use of Allopurinol for any given patient remains at the discretion of the clinical practitioner.**

6. References

- 6.1 HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol, Proc Natl Acad Sci U S A. 2005 Mar 15;102(11):4134-9

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