Texas BioGene, Inc.

HPAtype™ Platelet SSP HPA-1~6, 15 Typing Kit

Instructions for Use

REF 70030

Research Use Only

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1. INTENDED USE

This kit provides the Human Platelet Antigens (HPA) type 1~6, 15 typing results by a DNA-based method.

2. SUMMARY AND EXPLANATION

The human platelet antigens (HPA) are glycoproteins located on human platelets primarily responsible for mediating cellular adhesion upon vascular injury. In the presence of anti-HPA antibodies against self HPA, diseases such as neonatal alloimmune thrombocytopenia, post-transfusion purpura and platelet transfusion refractoriness can manifest and lead to hospitalization. Traditionally, HPA typing has been performed by serological methods. However, limitations of the traditional serological methodology become apparent as we become more knowledgeable about the HPA genes.

The HPA genes are polymorphic. As more DNA sequences of HPA alleles are revealed, it is clear that HPA antigens differ from each other by only a few or one single amino acid. Due to the limitation of the traditional serological methodology, the use of DNA-based methods is introduced to provide a higher typing resolution to differentiate these subtle antigenic differences. Texas BioGene would like to introduce the HPAtype™ Platelet SSP Typing Kit as a DNA-based method for the determination of HPA alleles for HPA-1~6, 15 types, using **s**equence **s**pecific **p**rimers (SSP).[3]

3. PRINCIPLE(S)

The HPAtype™ Platelet SSP HPA-1~6, 15 Typing Kit is for determining HPA alleles using PCR techniques with **s**equence **s**pecific **p**rimers (PCR-SSP). Allele sequence-specific primer pairs are designed to selectively amplify target sequences that are specific to a single allele. This PCR-SSP method is based on the principle that only primers with completely matched sequences to the target sequences result in amplified products under controlled PCR conditions. The presence of amplified DNA fragment is a positive indication of the existence of allele specific sequence within the genomic DNA. On the other hand, mismatched primers do not generate amplicons.

In addition to sequence specific primers, an internal control primer pair, which amplifies a conserved region of the house keeping gene, cystic fibrosis gene, is included in every PCR reaction mix, and the PCR product of the internal control primer pair serves as an indication of the integrity of PCR reaction. When the DNA fragment amplified by sequence specific primer is present, the product of the internal control primer pair is sometimes weak or absent due to the differences in amplifying efficiency between the specific primer pair and the internal control primer pair.

The PCR reaction products are examined by agarose gel electrophoresis that separates the DNA fragments by size. The amplified fragments are visualized as bands through ethidium bromide staining followed by ultraviolet light exposure.

The HPAtype™ Platelet SSP HPA-1~6, 15 Typing Kit supplies a panel of primer mixes that is designed to provide positive identification of HPA alleles. A worksheet is provided to interpret HPA genotypes by analyzing the resulting PCR products.

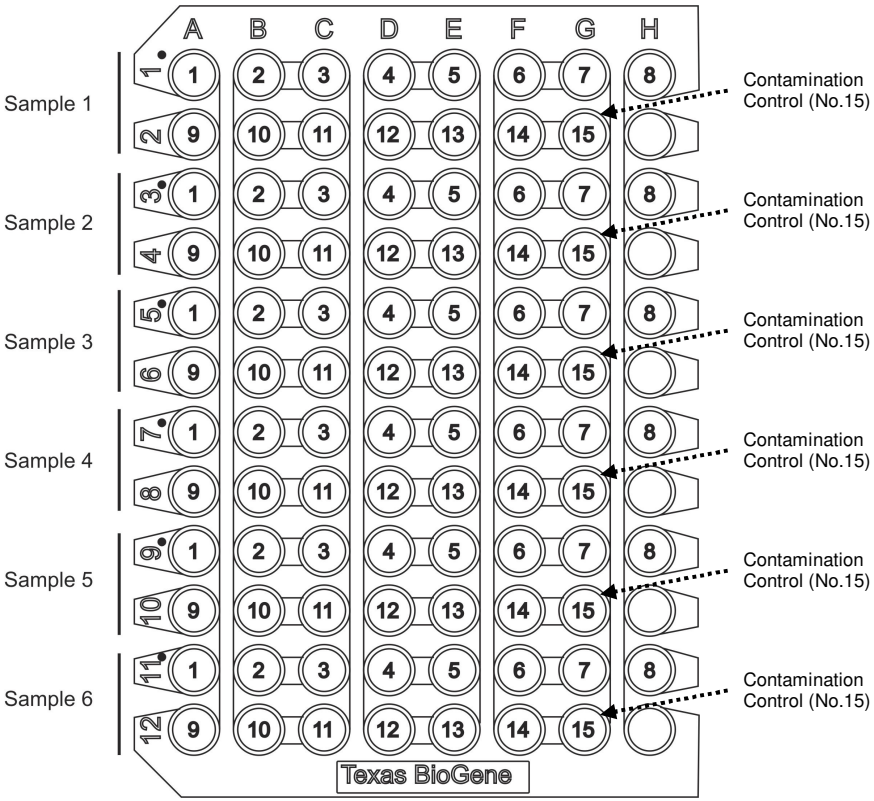
4. REAGENTS

4.1 Contents of the HPAtype™ Platelet SSP HPA-1~6, 15 Typing Kit

The HPAtype™ Platelet SSP HPA-1~6, 15 Typing Kit provides sufficient reagents for 12 tests.

4.1.1 Typing Trays

Each tray is designed for 6 tests, consisting of PCR tubes that contain dried primers. A black dot marks well one for each test. Tray layout with primer mix number is shown below.



HPA: 1~14 **Contamination Control: 15**

4.1.2 HPA Buffer 1020µl

The buffer is optimized for PCR reactions, and contains nucleotides, salts, glycerol and cresol red (final concentration: 0.15 mM dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, 15.75 mM Tris-HCl, 0.5 % glycerol). Each pre-aliquoted tube is for 6 tests.

4.1.3 PCR Plate Cover Membrane

The cover membrane is used to prevent evaporation loss during the PCR process.

4.1.4 Gel Interpretation Worksheets

The worksheet reveals the primer sets that are reactive to specific HPA alleles.

4.2 Warning or Caution

For Research Use Only

4.2.1 Do not use reagents past the expiration date printed on the label.

4.2.2 The test must be performed by well-trained and authorized laboratory staff.

4.2.3 Reagents should be handled in accordance with good laboratory practice using appropriate precautions.

4.2.4 Pipettes used for post-PCR manipulations should not be used for pre-PCR manipulations.

4.2.5 Biohazard Warning: the ethidium bromide used for staining of DNA is a potential carcinogen. Always wear gloves when handling stained gels.

4.2.6 Biohazard Warning: all blood products should be treated as potentially infectious.

4.2.7 Caution: wear UV-blocking eye protection and do not view UV light source directly when viewing or photographing gels.

See Material Safety Data Sheets for detailed information.

4.3 Storage

The Typing Trays are sealed in pouches and should be stored at 2-8°C. The HPA Buffer should be store at -20°C. Any unused Typing Tray should be returned to their original pouch and sealed using the zipper. Once the pouch is opened, the Typing Tray should be used within 4 weeks.

4.4 Instability Indications

Do not use Typing Trays with cracks in the tubes.

5. INSTRUMENT REQUIREMENTS

5.1 Programming the Thermal Cycler

The HPAtype™ Platelet SSP HPA-1~6, 15 Typing Kit is standardized for the following program using Applied Biosystems GeneAmp® 9600 and 9700. Thermal cyclers other than the recommended model and/or brand have to be user-validated. The program runs in approximately 1 hr 50 min.

Morgan HPA Program

Segment	Cycle Number	Temperature	Time
1	1	96°C	5 min
2	30	96°C	30 sec
		65°C	70 sec
		72°C	60sec
3	1	72°C	2 min
4	1	4°C	until removed

Please program the thermal cycler according to manufacture's instruction.

5.2 Gel Electrophoresis

Refer to Section 7.6 Gel Electrophoresis.

6. SPECIMEN COLLECTION AND PREPARATION

6.1 Blood Sample Collection

The peripheral blood can be collected using blood collection tubes with sodium citrate and EDTA as anticoagulants. Contamination of the DNA by heparin can result in interference with the PCR reaction. For this reason, heparinized blood should not be used as a starting material for DNA isolation. Please refer to manufacturer's instruction for blood sample storage and stability.

6.2 DNA Isolation

Genomic DNA may be obtained from all nucleated cells. The source of cell includes, but is not limited to, whole blood, buffy coat and cultured cells. DNA isolation can be performed by any validated protocol that produces DNA with adequate quality and quantity of DNA followed by repeated user validation.

6.3 DNA Quantity

The DNA sample should be re-suspended in sterile distilled water or appropriate buffer solution at a concentration of 10-40 ng/µl. DNA should not be re-suspended in solutions containing chelating agents, such as EDTA, above 0.5 mM in concentration.

6.4 DNA Quality

The DNA sample should have an A260/A280 ratio between 1.65 and 1.8.

DNA samples may be used immediately after isolation or stored at –20°C or below for extended periods of time (over 1 year) with no adverse effects on the HPA typing results.

7. DIRECTIONS FOR USE

7.1 Materials Provided

Refer to Section 4. REAGENTS

7.2 Materials Required, But Not Provided

7.2.1 PCR

7.2.1.1 Taq DNA polymerase (5 U/µl) (e.g. Texas BioGene Taq DNA polymerase, Cat. #38501~38504)
7.2.1.2 Distilled water or DNA diluent
7.2.1.3 Adjustable pipettes
7.2.1.4 Disposable pipette tips
7.2.1.5 Vortex mixer
7.2.1.6 Microcentirfuge
7.2.1.7 Table top centrifuge for 96 well PCR plates
7.2.1.8 Thermal Cycler with heated cover (e.g. Applied Biosystems GeneAmp 9600 and 9700)
7.2.1.9 Pressure pad

7.2.2 Gel Electrophoresis

7.2.2.1 Molecular biology grade agarose
7.2.2.2 0.5x TAE or TBE buffer
7.2.2.3 ethidium bromide solution (10 mg/ml)
7.2.2.4 Hot plate or microwave oven for heating agarose solutions
7.2.2.5 Gel tray and electrophoresis chamber Power supply
7.2.2.6 UV transilluminator
7.2.2.7 Photographic or image documentation system.

7.3 Sample Preparation

Refer to Section 6. SPECIMEN COLLECTION AND PREPARATION.

7.4 Reagent/Equipment Preparation

Refer to Section 5. INSTRUMENT REQUIREMENTS.

7.5 Performing PCR

7.5.1 Remove the tube of pre-aliquoted HPA Buffer from freezer and thaw at room temperature (20-25°C). Each tube is for 6 tests.

7.5.2 Once the HPA Buffer is thawed, vortex to mix well. Pulse-spin the buffer tube in a microcentrifuge to bring all liquid down to the bottom.

7.5.3 Remove Typing Trays from fridge. Take out the trays from the pouch. Return any unused tray to pouch and seal with the pouch zipper for future use.

7.5.4 Remove Taq DNA polymerase (5 U/µl) from freezer and keep on ice.

7.5.5 Combine 1 µl of Taq polymerase and 160 µl of HPA Buffer within a clean eppendorf tube. Cap tube and vortex to mix well. Pulse-spin in a microcentrifuge to bring all liquid down to the bottom.

7.5.6 Dispense 10 µl of the HPA Buffer/Taq polymerase mixture to the well #15, which is also the contamination control well.

7.5.7 To make the master mix, add 30 µl of DNA sample (10-40 ng/µl) to the remaining HPA Buffer/Taq polymerase mixture. Cap tube and vortex to mix well. Pulse-spin in a microcentrifuge to bring all liquid down to the bottom

7.5.8 Dispense 12 µl of the master mix into wells 1 through 14 of the Typing Tray. Usage of a repeater pipette is recommended. Care should be taken to avoid cross-contamination between wells by applying the master mix to the walls of the tube. Do not allow the pipette tips to contact the dried primer pellet at the bottom of each well.

7.5.9 Add 2 µl of distilled water or DNA diluent to the well #15.

7.5.10 Cover the Typing Tray with PCR Plate Cover Membrane. Make sure all tubes are properly covered by the membrane to prevent evaporative loss during the PCR process. Make sure the dispensed solution comes in contact with the dried primers on bottom of each tube by tapping or centrifugation.

7.5.11 Place a pressure pad on top of the PCR Plate Cover Membrane before closing the thermal cycler lid.

7.5.12 Close the heated thermal cycler lid and start the program shown in Section 5.1 of this manual.

7.5.13 After the PCR process is completed, you may proceed to gel electrophoresis immediately or store the tray at –20 °C and continue to gel electrophoresis at a later time.

7.6 Gel Electrophoresis

7.6.1 Gel Preparation

7.6.1.1 Prepare sufficient 2% agarose gel to resolve 15 PCR reactions according to the original gel electrophoresis system manufacture's instructions.

7.6.1.2 Make appropriate adjustments to ensure that the gel contains a final ethidium bromide concentration of 0.5 µg/ml.

7.6.1.3 Make sure that each well of the gel creates a minimum volume of 15 µl.

7.6.1.4 Make sure that the running buffer used for electrophoresis is consistent with the buffer used for gel preparation.

7.6.2 Performing Gel Electrophoresis

7.6.2.1 Transfer the entire volume of each PCR reaction mix (12 µl) into the wells in the proper sequence. A black dot marks the position one for each test and the order of samples is from left to right, top to bottom.

7.6.2.2 The electrophoresis takes place at 10V/cm until the cresol red has migrated about 0.7-1.0 cm into the gel.

7.6.2.3 After the electrophoresis is completed, place the agarose gel on an UV transilluminator. Photograph for documentation and HPA type interpretation.

8. Results

8.1 Data Analysis

8.1.1 The DNA fragments amplified by allele specific primer pairs are designed to have a size in the range between 150~600 bp. Refer to the worksheet for the exact size of the specifically amplified DNA fragment in each reaction tube. The internal control fragment is 945 bp in size. Therefore, the internal control band is the slower migrating band compared to the allele specific band. The presence of the allele specific band is scored as positive for the particular reaction. The absence of this allele specific band is scored as negative.

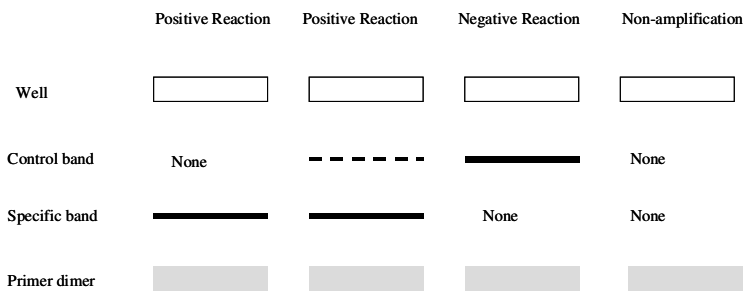
8.1.2 The internal control band may be weak or absent when the allele specific band is present. As the presence of the specific band is also an indication to the success of the PCR reaction. This is not a limitation to the test.

8.1.3 The internal control band should always be visible in negative lanes. Absence of both the internal control band and allele specific band indicates failed reaction and can void the test result.

8.1.4 The presence of a band in the contamination control well voids the test result.

8.1.5 Document the positive reactions for the test and determine the HPA types by using the worksheet provided.

8.2 Gel Interpretation



8.3 Limitation of Procedure

8.3.1 Performance of the test can only be guaranteed if the enclosed instructions are strictly followed.

8.3.2 All instruments, including the thermal cycler and pipetting devices, must be calibrated according the manufacturer's instructions.

8.3.3 The HPAtype™ Platelet SSP HPA-1~6, 15 Typing Kit is standardized for the Taq polymerase from Texas BioGene. The use of other brands of Taq polymerase may result in non-specific false positive PCR reactions.

8.3.4 The quality and quantity of the DNA samples have to be within the range as specified within instruction manual.

8.3.5 The results obtained from the HPAtype™ Platelet SSP HPA-1~6, 15 Typing Kit should for research use only.

8.4 Quality Control

Each manufactured lot is checked against a panel of standard DNA samples. The alleles carried by these DNA samples react with the corresponding primer mixes in the kit. The report is available upon request.

9. PERFORMANCE CHARACTERISTICS

9.1 Reproducibility

Ten standard DNA samples from International Histocompatibility Working Group (IHWG) were typed using the HPAtype™ Platelet SSP HPA-1~6, 15 Typing Kit for 5 times for the within-lot and inter-lot reproducibility studies. The data show 100% reproducibility for HPA alleles.

10. TROUBLESHOOTING

Problem	Potential Cause	Solution
Weak bands or absence of bands	Poor DNA quality	Repeat DNA purification and make sure the A260/A280 ratio is between 1.65-1.80.
	Insufficient amount of DNA	Make sure the DNA concentration is within the suggested range of 10~40ng/μl.
	Insufficient Taq polymerase activity	Make sure there is sufficient amount of Taq polymerase added and the Taq polymerase has not been degraded.
	Improperly calibrated thermal cycler	Calibrate the thermal cycler.
	Unevenly mixed PCR master mix	Make sure the master mix is thoroughly mixed before use.
	Insufficient ethidium bromide staining	Re-stain the agarose gel in fresh 0.5X TBE buffer with 0.5 μg/ml ethidium bromide.
Presence of false positive band(s)	Overly high DNA concentration	Make sure the DNA concentration is within the suggested range of 10~40ng/μl.
	Excessive Taq polymerase activity	Make sure the amount of Taq polymerase added is as instructed on page 8.
	Improperly calibrated thermal cycler	Calibrate the thermal cycler.
Band(s) in the contamination control well	Reagent contamination	Use new reagents for PCR preparation.

11. BIBLIOGRAPHY

1. Kroll, H., Kiefel, V., and Santoso, S. Clinical aspects and typing of platelet alloantigens. Vox Sang. 74 (Suppl 2):345-354, 1998.
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3. Newton, C.R., Graham, A., Heptinstall, E., Powell, S.J., Summers, C., Kalsheker, N., Smith, J.C., and Markham, A.F. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Research 17:2503-2516, 1989.

12. TRADEMARKS USED IN THIS DOCUMENT

HPAtype™
Texas BioGene, Inc.
Applied Biosystems
GeneAmp®

13. PATENTS USED IN THIS DOCUMENT

This product is optimized for use in the Polymerase Chain Reaction ("PCR") Process which is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd ("Roche"). No license under these patents to use the PCR Process is conveyed expressly or by implication to the purchaser by the purchase of this product. Further information on purchasing licenses to practice PCR may be obtained by contacting, in the United States, the Director of Licensing at Roche Molecular Systems, Inc. 1145 Atlantic Avenue, Alameda, California 94501, and outside the United States, the PCR Licensing Manager, F. Hoffmann-La Roche Ltd, Grenzacherstr. 124, CH-4070 Basel, Switzerland.

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