TBG Biotechnology Corp.

ExProbe™ SE HPA 1~6, 15, 21 Typing Kit

Instructions for Use

REF 67020

RUO

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Table of Contents

1. INTENDED USE

This kit provides the Human Platelet Antigens (HPA) typing results by performing real-time PCR 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 ExProbe^{TM} SE HPA 1~6, 15, 21 Typing Kit as DNA-based method for the determination of HPA genotypes for HPA-1~6, 15 types, using sequence specific primers (SSP) by real time PCR.

System	Glycoprotein	Antigen	Original name	Nucleotide change
HPA-1	GPIIIa	HPA-1a	Zw^{a}, Pl^{A1}	T^{176}
		HPA-1b	Zw^{b}, Pl^{A2}	C^{176}
HPA-2	GPIb	HPA-2a	Ko^{b}	C^{482}
		HPA-2b	Ko^{a}, Sib^{a}	T^{482}
HPA-3	GPIIb	HPA-3a	Bak^{a}, Lek^{a}	T^{2621}
		HPA-3b	Bak^{b}	G^{2621}
HPA-4	GPIIIa	HPA-4a	Yuk^{b}, Pen^{a}	G^{506}
		HPA-4b	Yuk^{a}, Pen^{b}	A^{506}
HPA-5	GPIa	HPA-5a	Br^{b}, Zav^{b}	G^{1600}
		HPA-5b	Br^{a}, Zav^{a}, Hc^{a}	A^{1600}
HPA-6	GPIIIa	HPA-6a	-	G^{1544}
		HPA-6b	Ca^{a}, Tu^{a}	A^{1544}
HPA-15	CD109	HPA-15a	Gov^{b}	C^{2108}
		HPA-15b	Gov^{a}	A^{2108}
HPA-21	GPIIIa	HPA-21a	-	G^{1960}
		HPA-21b	Nos	A^{1960}
Reference: http://www.ebi.ac.uk/ipd/hpa/				

3. PRINCIPLE(S)

The ExProbe^{TM} SE HPA 1~6, 15, 21 Typing Kit is for determining HPA genotype using real-time PCR (RT-PCR) techniques containing a panel of primer mixes and fluorescent probes. This method is based on sequence specific primers that completely match the target sequences resulting in DNA amplification. The presence of amplification is detected by activation of fluorescent probe(s) and a positive indication of the existence of allele specific sequence within the genomic DNA. On the other hand, mismatched primers do not result in amplification and thereby do not emit fluorescent signal(s).

An internal control primer pair that amplifies a conserved region of the house keeping gene, cystic fibrosis gene, is included in every RT-PCR reaction mix, and the PCR product of the internal control primer pair serves as an indication of the integrity of PCR reaction.

For more information about the real-time PCR method, refer to “About Real-time PCR Primer and Probe chemistry” supplement document.

4. REAGENTS

4.1 Contents of the ExProbe™ SE HPA 1~6, 15, 21 Typing Kit

ExProbe™ SE HPA 1~6, 15, 21 Typing Kit provides sufficient reagents for 12 tests.

4.1.1 PP mixes

The typing kit contains 16 PP mixes. Each PP mix contains the primers to amplify the specific HPA alleles and the probes to detect the presence of amplification.

4.1.2 RP Buffer

The buffer is optimized for PCR reactions, and contains nucleotides, salts, glycerol (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 RPTaq polymerase (5 U/µl)

Each tube of RPTaq is used to amplify the HPA alleles during the amplification process.

4.1.4 Positive control DNA

Positive control DNA can be amplified by PP mix #16 during the amplification process as a positive control.

4.2 Warning or Caution

For Research Use

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

See Material Safety Data Sheets for detailed information.

4.3 Storage

The Typing Kits should be stored at -20°C. Once opened, any unused reagents should be returned to the typing kit box and store at -20°C afterwards.

4.4 Instability Indications

Do not use Typing Kits with cracks in the tubes.

5. INSTRUMENT REQUIREMENTS

5.1 Programming the Real Time Thermal Cycler

The ExProbe™ SE HPA 1~6, 15, 21 Typing Kit is standardized for the following program using Applied Biosystems® 7500 Real-Time PCR Systems.

Please make sure the system has been properly calibrated for the dye(FAM , Texas Red and Hex).

Real time thermal cyclers other than the recommended model and/or brand have to be user-validated. The program runs in approximately 1 hr.

RP Program

Segment	Cycle Number	Temperature	Ramp Rate	Time
1	1	96ºC	100%	2 min
2	35	94ºC	100%	10 sec
		65ºC (Data Collection)	100%	45 sec

Detection Format

Target	Reporter	Quencher
HPA1-6,15	FAM	None
HPA21	Texas Red	None
IC	Hex	None

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

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 5-40ng/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 1.9.

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 Distilled water or DNA diluent
7.2.1.2 Adjustable pipettes
7.2.1.3 Disposable pipette tips
7.2.1.4 Vortex mixer
7.2.1.5 Microcentirfuge
7.2.1.6 Table top centrifuge for PCR plates
7.2.1.7 Real-time PCR detection system (e.g. Applied Biosystems® 7500)

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 Thaw all required reagents completely and put them on ice. Mix all reagents well and spin them down prior to pipetting. Ensure that the PP mixes are exposed to as little light as possible.

7.5.2 Dispense 8µl of each PP mix into the 96-Well Plate/8-Tube Strip according to the Figure 1.

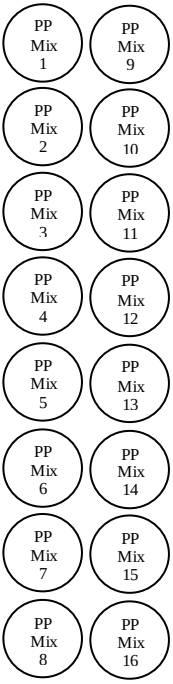

Figure 1. The position of PP Mixes on plate/strip

7.5.3 Combine 1.8 µl of RPTaq polymerase and 135 µl of RP 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.

7.5.4 Dispense 7 µl of the RP Buffer/RPTaq polymerase mixture into the PP Mix15 and PP Mix16 well.

7.5.5 Add 2 µl of distilled water or DNA diluent to the PP Mix15 well which is the contamination control well.

7.5.6 Add 2 µl of positive control DNA to the PP Mix16 well which is the positive control well.

7.5.7 To make the master mix, add 32.5 µl of DNA sample (5-40 ng/µl) to the remaining RP Buffer/RPTaq 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 9 µl of the master mix into PP mix 1 through PP mix 14 well. 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.

7.5.9 Seal the PCR plate/8-Tube Strip with Optical adhesive film/Cap. Centrifuge at 1,200 rpm for 30sec.

7.5.10 Place the plate/strip in the real-time PCR instrument, and run the program shown in Section 5.1 of this manual.

8. Results

8.1 Data Analysis settings:

Target	Reporter	Threshold	Baseline
HPA1-6,15	FAM	25,000	3-15
HPA21	Texas Red	35,000	3-15
IC	Hex	5,000	3-15

8.2 Data interpret table:

PP Mix #	Acceptable IC C_T Range	HPA1-6,15 Target C_T cut off	HPA21 Target C_T cut off	Genotype	
1	20-27	≦33	≦33	HPA-1a	HPA-21a
2	20-27	≦33	≦33	HPA-1b	HPA-21b
3	20-27	≦33	NA	HPA-2a	
4	20-27	≦33	NA	HPA-2b	
5	20-27	≦34	NA	HPA-3a	
6	20-27	≦34	NA	HPA-3b	
7	20-27	≦33	NA	HPA-4a	
8	20-27	≦33	NA	HPA-4b	
9	20-27	≦33	NA	HPA-5a	
10	20-27	≦33	NA	HPA-5b	
11	20-27	≦33	NA	HPA-6a	

12	20-27	≦33	NA	HPA-6b
13	20-27	≦33	NA	HPA-15a
14	20-27	≦33	NA	HPA-15b
15	≧30*	NA	NA	Contamination control
16	20-27	≦33	NA	Positive control

*If the IC C_T value for well 15, the contamination control well, is less than 30, the reaction is contaminated and the result is voided (See section 8.2.3).

8.2.1 If IC C_T exceeds the acceptable range, the result is voided and refers to section 10 Troubleshooting.

8.2.2 If no a and b subtypes for the same locus, the result is voided and refer to section 10 Troubleshooting.

8.2.3 If the IC C_T of Well #15 is ≦30 or the target C_T of Well#16 >33 the result is voided and refer to section 10 Troubleshooting.

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 real time thermal cycler and pipetting devices, must be calibrated according the manufacturer's instructions.

8.3.3 The ExProbe™ SE HPA 1~6, 15, 21 Typing Kit is standardized for the RPTaq polymerase from TBG. 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 ExProbe™ SE HPA 1~6, 15, 21 Typing Kit should be 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 Specific Performance

Performance was evaluated by testing 50 random whole blood specimens and comparing with HPA genotype sequencing results. The results showed 100% concordance (350/350) for the HPA genotypes as summarized in the following table.

Genotypes	Number of Concordant Tests	Number of Tests	Concordance	95 % Confidence Limits	
				Lower	Upper
HPA-1	50	50	100%	100%	100%
HPA-2	50	50	100%	100%	100%
HPA-3	50	50	100%	100%	100%
HPA-4	50	50	100%	100%	100%
HPA-5	50	50	100%	100%	100%
HPA-6	50	50	100%	100%	100%
HPA-15	50	50	100%	100%	100%
HPA-21	50	50	100%	100%	100%
Total	350	350	100%	100%	100%

9.2 Quality Control of Kit Manufacturing

Ten standard DNA samples from International Histocompatibility Working Group (IHWG) were typed using ExProbe™ SE HPA 1~6, 15, 21 Typing Kit for 5 times for the within-lot and inter-lot reproducibility studies. The data show 100% reproducibility for HPA alleles.

9.3 Reproducibility

Ten standard DNA samples from International Histocompatibility Working Group (IHWG) were typed using the ExProbe™ SE HPA 1~6, 15, 21 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
IC C_T exceeds acceptable range	Poor DNA quality	Repeat DNA purification and make sure the A260/A280 ratio is between 1.65-1.9.
	Amount of DNA exceeds acceptable range	Make sure the DNA concentration is within the suggested range of 5-40ng/µl.
	Insufficient RPTaq polymerase activity	Make sure the RPTaq polymerase has not been degraded.
	Improperly calibrated real time thermal cycler	Calibrate the real time thermal cycler.
	Unevenly mixed PCR master mix	Make sure the master mix is thoroughly mixed before use.
No a and b subtypes for the same locus. Or Target C_T of Well#16 >33	Insufficient DNA concentration	Make sure the DNA concentration is within the suggested range of 5-40ng/µl.
	Insufficient RPTaq polymerase activity	Make sure the amount of RPTaq polymerase added is as instructed on page 7.
	Improperly calibrated real time thermal cycler	Calibrate the real time thermal cycler.
IC C_T of Well #15 is $\leqq$30	distilled water or DNA diluent contamination	Use new aliquots of distilled water or DNA diluent for PCR preparation
	Reagent contamination	Use new aliquots of reagents for PCR preparation

11. BIBLIOGRAPHY

1. Cruse JM and Lewis RE, 1998, Atlas of Immunology. CRC Press: 77-97
2. 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

TBG Biotechnology Corp.
Roche LightCycler®
ExProbe™

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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