

## PAI-1 4G/5G REAL-TIME PCR KIT

### Cat. No: 108R-10-01

#### INTRODUCTION

A decreased fibrinolytic activity due to increased levels of plasminogen activator inhibitor-1 (PAI-1) has been shown in patients suffering deep vein thrombosis. Elevated plasma PAI-1 levels are associated with the 4G allele of a 4G/5G insertion/deletion polymorphism located in the promoter region 675 bp upstream from the transcription start sequence of the PAI-1 gene <sup>(1)</sup>.

#### INTENDED USE

PAI-1 4G/5G Real-Time PCR Kit can detect PAI-1 5G/4G polymorphism of PAI-1 gene in whole blood samples by using qualitative Real-Time PCR method.

#### TARGETED USER

For professional use only. Testing should be performed by professionals trained in molecular techniques.

#### PRINCIPLE OF THE SYSTEM

During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes perfectly to the target DNA. This cleavage results in the fluorescent signal which is monitored by Real-Time PCR detection system. An increase in the fluorescent signal ( $C_T$ ) is proportional to the amount of the specific PCR product <sup>(2,3)</sup>.

#### PRODUCT SPECIFICATION

Each isolated DNA should be tested with two master mixes separately. The kit provides reagents in a "ready-to-use" master mix format which has been specifically adapted to 5' nuclease PCR for SNP analysis. The test system is designed by SNP Biotechnology for use with sequence specific primers and probes.

The fluorescence of polymorphism analysis is FAM. Also each master mix contains an internal control labelled with HEX/JOE dye. Internal Control is Zinc Finger Protein Gene, X Linked – ZFX (OMIM: 314980).

The limit of detection (LOD) for the PAI-1 4G/5G Real-Time PCR Kit was determined as 1 ng/μl.

#### SYSTEM CONTENTS

| Reagents            | 10 rxns | 20 rxns | 50 rxns |
|---------------------|---------|---------|---------|
| PAI-1 4G Master Mix | 200 μl  | 400 μl  | 1000 μl |
| PAI-1 5G Master Mix | 200 μl  | 400 μl  | 1000 μl |
| Control DNA*        | 30 μl   | 30 μl   | 60 μl   |

**Table 1:** Kit content

\* Control DNA is a synthetic plasmid containing the polymorphism regions. Expected results for synthetic control DNA should be PAI-1 5G/4G. Since to Control DNA is a synthetic plasmid, amplification plots of synthetic control DNA may appear slightly different from the sample DNA. Please gently vortex and then spin centrifuge for 1-2 seconds before using the positive control.

#### STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expiration date on the box label.
- Repeated thawing and freezing (>4X) should be avoided, as this may reduce the sensitivity of the assay.

#### SAMPLE COLLECTION

PAI-1 4G/5G Real-Time PCR Kit is approved for use with whole blood samples.

- Standard precautionary instructions must be followed by all healthcare professionals during the collection and transportation of whole blood samples.
- Whole blood samples should be collected in appropriate containers before delivery to the laboratory.
- Freezing and thawing of samples should be avoided.

#### DNA EXTRACTION

Blood samples should be collected in appropriate sterile EDTA tubes and can be stored at +4°C up to one month. For more than one month specimen should be stored at -20°C. It is advised to gently mix the tube (with EDTA) after collection of blood to avoid coagulation.

Our system optimized according to GeneAII® Exgene™ Blood SV. It is advised to elute DNA with 150 μl elution buffer for better results.

#### PROCEDURE

- Different 4G and 5G tubes should be prepared for each master mix.
- Leave the master mixes\* and controls at RT to melt.
- Before starting work, mix the master mixes gently by pipetting
- For each sample, pipet **20 μl master mix** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **5 μl DNA** into each tube. Please do not pipette DNA before and after addition into well.
- Optical caps are closed, it is recommended to spin the plates/strips at low speed for a short time.
- Run with the programme shown below.

\*Master mixes include HotStart Taq DNA Polymerase.

#### PCR PROGRAMME

|       |         |           |
|-------|---------|-----------|
| 95 °C | 3 Min.  | Holding   |
| 95 °C | 15 Sec. | 30 Cycles |
| 60 °C | 1 Min.  |           |

**Table 2:** PCR Programme

Fluorescent dyes are FAM and HEX/JOE.

#### **This system can be used with the following devices:**

- Bio-Rad CFX96
- ABI Prism® 7500/7500 Fast
- Roche LightCycler® 480 System
- Rotor Gene Q
- Mic qPCR Cycler

For other two or more channel Real-Time PCR devices (which can read FAM and HEX/JOE dyes), a trial run is recommended.

#### **If you use:**

ABI Prism® system, please choose "none" as passive reference and quencher.

Mic qPCR Cycler, please adjust gain settings, "Green Auto Gain" to 20 and "Yellow Auto Gain" to 10

#### Supplied Materials

- White PCR plates/strips with optical covers\*

\*The PCR Plate/strip tube and caps seriously affect the amplification curve quality. Therefore, white PCR plates/strips and optical caps provided by the manufacturer should be used with the kit.

#### Required Materials (Not Provided)

- PCR Cabinet
- Vortex Mixer
- Desktop Microcentrifuge (For 2.0ml tubes and PCR strip tubes), plate spin for studies using PCR plates.
- Automated or spin column based DNA isolation Kit
- Disposable powder-free laboratory gloves
- Micropipettes (0.5ml-1000ml)
- Micropipette tips
- Standard laboratory equipments.

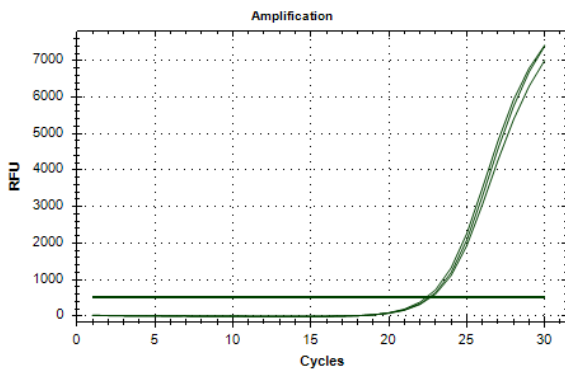
#### DATA ANALYSIS

After the run is completed data are analysed using the software with FAM and HEX/JOE dyes. The below results were studied with Bio-Rad CFX96. The threshold values for all dyes were set to 500, based on experiments conducted using the Bio-Rad CFX96 Real-Time PCR system, the GeneAll® Exgene™ Blood SV Isolation Kit, and white PCR strips supplied by SNP Biotechnology. Threshold values may vary depending on the PCR device, DNA isolation kit, and the type or brand of PCR strips/tubes used.

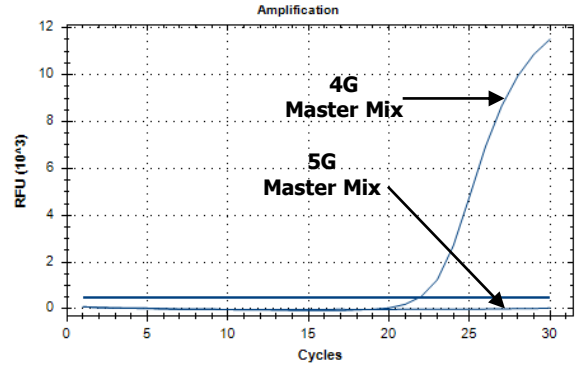
Internal control amplification plots must be seen in all wells except NTC and has been labelled with HEX/JOE dye. The  $C_T$  value of internal controls should be  $21 \leq C_T \leq 28$ . These values are optimised according to the GeneAll® Exgene™ Blood SV Isolation Kit and Bio-Rad CFX96 Real-Time PCR Device.  $C_T$  values may vary  $\pm 2/3$  cycle according to the other DNA isolation systems and Real-Time PCR devices.

Amplification plots of polymorphism can be analysed by FAM dye. The  $C_T$  value should be between  $21 \leq C_T \leq 28$ . These values are optimised according to the GeneAll® Exgene™ Blood SV Isolation Kit and Bio-Rad CFX96 Real-Time PCR Device.  $C_T$  values may vary  $\pm 2/3$  cycle according to the other DNA isolation systems and Real-Time PCR devices.

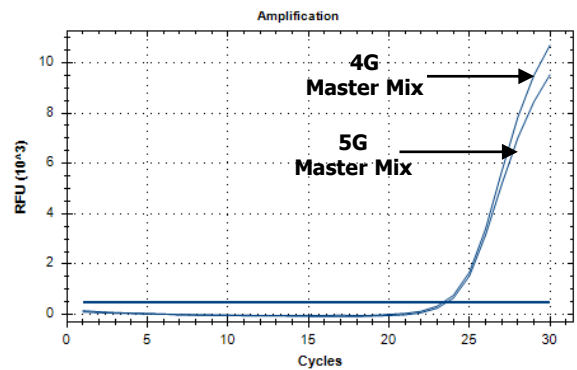
- Homozygote 5G sample gives amplification signal only with 5G master mix.
- Heterozygote 5G/4G sample gives amplification signal both with 5G and 4G master mixes.
- Homozygote 4G sample gives amplification signal only with 4G master mix.
- The difference of the  $C_T$  value with 5G and 4G amplification plots should be  $\leq 3$  for heterozygote sample. If it is  $4 \leq C_T \leq 6$ , test should be repeated, if  $>6$ , the late plot should be considered as non-specific.



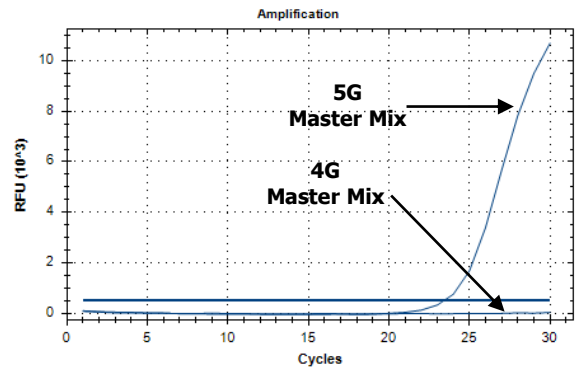
**Figure 1:** Internal Control plots – HEX/JOE Dye



**Figure 2:** 4G/4G Homozygous Sample (FAM Dye)



**Figure 3:** 4G/5G Heterozygous Sample (FAM Dye)



**Figure 4:** 5G/5G Homozygous Sample (FAM Dye)

#### CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR master mixes forgotten at room temperature.
- Thaw PCR master mix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR master mixes is 12 months. Please check the manufacturing data before use.
- Only use in vitro diagnostics.

## DISPOSAL OF KIT

Dispose of it according to the legal regulations of your region

## REFERENCES

1. Maurizio Margaglione, Giuseppe Cappucci, Donatella Colaizzo, Nicola Giuliani, Gennaro Vecchione, Elvira Grandone, Orazio Pennelli and Giovanni Di Minno. "The PAI-1 Gene Locus 4G/5G Polymorphism Is Associated With a Family History of Coronary Artery Disease". Arterioscler Thromb Vasc Biol. 1998 Feb;18(2):152-6.
2. Yolanda S Lie and Christos J Petropoulos. "Advances in quantitative PCR technology: 5' nuclease assays". Current Opinion in Biotechnology Volume 9, Issue 1, February 1998, Pages 43-48.
3. Luis Ugozzoli and R. Bruce Wallace. "Allele-Specific Polymerase Chain Reaction". A Companion to Methods in Enzymology Vol. 2, No. 1, February, pp. 42-48, 1991.

## SYMBOLS AND DESCRIPTIONS

|                                                                                   |                                |                                                                                     |                                                                                        |
|-----------------------------------------------------------------------------------|--------------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
|  | Catalog Number                 |  | CE Mark                                                                                |
|  | Lot Number                     |  | Unique Device Identifier<br>(01)Device Identifier<br>(17)Expiry Date<br>(10)Lot Number |
|  | Manufacturer                   |  | Test Quantity                                                                          |
|  | Fragile                        |  | Storage Temperature                                                                    |
|  | Protect from directly sunlight |  | In Vitro Diagnostics                                                                   |
|  | Expiry Date                    |                                                                                     |                                                                                        |

**Table 3:** Symbols and descriptions

## TROUBLESHOOTING PROBLEMS AND SOLUTIONS

| Problem                                                                                                           | Reason                                                               | Solution                                                                                                           |
|-------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|
| Internal control does not work/ low amplification                                                                 | Absence of DNA / DNA extraction problems                             | Repeat test                                                                                                        |
|                                                                                                                   | Absence of DNA / DNA extraction problems                             | • DNA extraction should be repeated.                                                                               |
|                                                                                                                   | Sample is containing PCR inhibitor(s)                                | • DNA extraction should be replaced with one of the recommended methods.                                           |
| No target gene amplification curves in some samples for both 5G and 4G mixes.                                     | Absence of DNA / not added into well                                 | Repeat test                                                                                                        |
|                                                                                                                   | Absence of DNA / DNA extraction problems                             | • DNA extraction should be repeated.                                                                               |
|                                                                                                                   | Sample is containing PCR inhibitor(s)                                | • DNA extraction should be replaced with one of the recommended methods.                                           |
| No target DNA/internal control amplification curves in all wells                                                  | Error in temperature/time settings in PCR program                    | Correct any errors in the temperature/time settings in the PCR Program and repeat the test.                        |
|                                                                                                                   | Sample is containing PCR inhibitor(s)                                | • DNA extraction should be repeated.<br>• DNA extraction should be replaced with one of the recommended methods.   |
| Positive control result and/or C <sub>T</sub> values are lower or higher than the value mentioned in User Manual. | Error in temperature/time settings in PCR program                    | Correct any errors in the temperature/time settings in the PCR Program and repeat the test.                        |
| C <sub>T</sub> values are not valid (higher or lower) according to User Manual                                    | Excessive or insufficient DNA sample                                 | • Repeat the test.<br>• DNA extraction should be repeated.                                                         |
| Low and/or invalid amplification curves                                                                           | Stability problems arising from repeated thawing and freezing ( >4X) | Repeated thawing and freezing ( >4X) should be avoided, as this may reduce the sensitivity of the assay.           |
|                                                                                                                   | Sample is containing PCR inhibitor(s)                                | • DNA extraction should be repeated.<br>• DNA extraction should be replaced with one of the recommended methods.   |
|                                                                                                                   | Stability problems arising from unavailable storage conditions.      | All reagents should be stored at – 20 °C and dark.                                                                 |
|                                                                                                                   | Bubble formation or pipetting error during pipetting                 | After adding the master mix and sample, it is recommended to spin the plates/strips at low speed for a short time. |
| For further questions, please contact us <a href="mailto:tech@snp.com.tr">tech@snp.com.tr</a>                     |                                                                      |                                                                                                                    |

**Table 4:** Troubleshooting problems and solutions