“Pharmacogenetics”
Real-time PCR based SNP genotyping assay

Warfarin Pharmacogenetics
REAL-TIME PCR Genotyping Kit

General information

Overview:
“Pharmacogenetics” Real-time PCR based SNP genotyping assay allows detection of a range of genetic polymorphisms associated with individual response to pharmaceuticals. The employed Real-time PCR method discriminates alleles thus providing genotype information that helps to estimate odds of adverse effects associated with drug administration, or to predict efficiency of therapy. This assay includes the following kits:

Warfarin Pharmacogenetics REAL-TIME PCR genotyping Kit is intended for detection and allelic discrimination of genetic polymorphisms associated with an individual’s response to Coumarin (Warfarin) therapy. The results of the genetic test can be used for assessment of effective and safe dosage of Coumarin (Warfarin).

Method:
Real-time PCR followed by melting curve analysis, qualitative analysis.

Samples:
Peripheral blood.

DNA extraction:
The “DNA-Technology” PREP-GS Genetics or PREP-RAPID Genetics kits are recommended for DNA extraction.

Features:
Two alleles are detected simultaneously in single tube.
PCR-Mix contains an internal control (DNA-IC). IC is intended for PCR quality and sufficiency of DNA assurance.
We also recommend including in assay the negative control (“C–“) which is not supplied but very helpful for contamination control purposes. Use deionized water or sterile buffered saline instead of sample, starting from extraction step.

Devices:
The automatic analysis for given REAL-TIME PCR Genotyping Kits is available on “DNA-Technology” made DTitel, DTprime and DT-96 REAL-TIME Thermal Cyclers; software version is not lower than 7.3.5.57; the latest version of the software is available for download at http://www.dna-technology.ru/eng/support/

Please enquire DNA-Technology company’s representative about compatibility of third-party Real-time instruments.

Overall time needed to perform the analysis (excluding sample preparation procedure):
2 hours at average.

The number of tests:
48

1 - supported by 4S1; 4S2; 5S1; 5S2; 6S1; 6S2 instruments
2 - supported by 4M1; 4M3; 4M6; 5M1; 5M3; 5M6; 6M1; 6M3; 6M6 instruments
Reagent | Quantity | PCR-mix
--- | --- | ---
1. CYP2C9: 430 C>T (Arg144Cys) | 960 μL | 1 tube
2. CYP2C9: A>C (Ile359Leu) | 960 μL | 1 tube
3. CYP4F2: C>T (Val433Met) | 960 μL | 1 tube
4. VKORC1: -1639 G>A | 960 μL | 1 tube
PCR-buffer | 960 μL | 2 tubes
Taq-AT-polymerase | 96 μL | 1 tube
Mineral oil | 3.84 mL | 1 vial

**Dye label detection channels corresponding to allelic variants and IC**

<table>
<thead>
<tr>
<th>PCR-mix</th>
<th>Fam</th>
<th>Hex</th>
<th>Rox</th>
<th>Cy5</th>
<th>Cy5.5</th>
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<tr>
<td>CYP2C9: 430 C&gt;T (Arg144Cys)</td>
<td>C</td>
<td>T</td>
<td>-</td>
<td>IC</td>
<td>-</td>
</tr>
<tr>
<td>CYP2C9: A&gt;C (Ile359Leu)</td>
<td>A</td>
<td>C</td>
<td>-</td>
<td>IC</td>
<td>-</td>
</tr>
<tr>
<td>CYP4F2: C&gt;T (Val433Met)</td>
<td>C</td>
<td>T</td>
<td>-</td>
<td>IC</td>
<td>-</td>
</tr>
<tr>
<td>VKORC1: -1639 G&gt;A</td>
<td>G</td>
<td>A</td>
<td>-</td>
<td>IC</td>
<td>-</td>
</tr>
</tbody>
</table>

### Procedure

1. **PCR amplification**

   The quantity of DNA to be analyzed must be greater than or equal to 1.0 ng per reaction (the Cp parameter for IC must not be more than 32.0). The violation of this requirement will affect the validity of analysis and void the manufacturer guarantee.

   1.1 Mark the required number of 0.2 mL PCR-tubes for each polymorphism to be tested (one tube for each sample to be tested and one extra for negative control "C-").

   **Example:** for simultaneous testing of 5 samples in one PCR run, mark 20 tubes for samples and 4 tubes for "C-". The resulting number of tubes is 24 (see Table 1).

   **Table 1. PCR tubes marking for 5 samples testing.**

<table>
<thead>
<tr>
<th>CYP2C9: 430 C&gt;T (Arg144Cys)</th>
<th>CYP2C9: A&gt;C (Ile359Leu)</th>
<th>CYP4F2: C&gt;T (Val433Met)</th>
<th>VKORC1: -1639 G&gt;A</th>
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</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Sample 2</td>
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<tr>
<td>Sample 3</td>
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<td>✓</td>
</tr>
<tr>
<td>Sample 4</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Sample 5</td>
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</tr>
<tr>
<td>&quot;C-&quot;</td>
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<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

1.2 Vortex the tubes for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

1.3 Add 20 μL of corresponding PCR-mix into the marked tubes (use a new pipette tip for each type of PCR-mix).

1.4 Vortex the tubes with PCR-buffer and Taq-AT-polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

**Taq-AT-polymerase must be stored at minus 20 °C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.**

1.5 Prepare the mixture of PCR-buffer and Taq-AT-polymerase. Add into one tube:

   - 10×(N+1) μL of PCR-buffer;
   - 0.5×(N+1) μL of Taq-AT-polymerase;
   - N — number of the marked tubes including "C-" (see Table 1).

**Example:** For simultaneous testing of 5 samples and 1 "C-" (resulting number of marked tubes is 24), prepare mix of PCR-buffer and Taq-AT-polymerase for 25 (24+1) tubes, i.e. mix 250 μL of PCR-buffer with 12.5 μL of Taq-AT-polymerase.
1.6 Vortex the tube for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

The mixture of PCR-buffer and Taq-AT-polymerase must be prepared just prior to use.

1.7 Add 10 μL of PCR-buffer and Taq-AT-polymerase mixture into each PCR-tube.

1.8 Add one drop (~20 μL) of mineral oil in each PCR-tube. Close the tubes tightly.

Follow the steps listed in 1.9 - 1.13 within two hours after addition of PCR-buffer and Taq-AT-polymerase mix to amplification mix.

1.9 Add 5.0 μL of DNA sample into corresponding PCR-tubes. Open the tube, add DNA sample, then close the tube before proceeding to the next DNA sample to prevent contamination. Use filter tips. Do not add DNA into the “C-” tubes.

1.10 Add 5.0 μL of negative control (“C-“) which passed whole DNA extraction procedure into corresponding tubes.

1.11 Spin the tubes for 1-3 seconds to collect the drops.

1.12 Set the tubes to Real-time PCR instrument.

1.13 Launch the Real-time_PCR application in «Device handling mode». Upload the ini file «Warfarine.ini» before the first run. Add tests «CYP2C9:__430_C>T», «CYP2C9:__A>C», «CYP4F2:__C>T» and «VKORC1:__1639_G>A» or use multiple test mode in subsequent runs. Specify the number and types of samples including negative controls. Define position of strips in the software interface according to position they were set in the thermoblock (see. 1.12). Run PCR.

The type of the negative control tubes must be specified as “Sample”.

2 Registration and interpretation of the PCR results are operated by software and held in automatic mode.

For samples containing a sufficient quantity of DNA for correct analysis, the software defines the genotype. The samples containing an insufficient quantity of DNA (less than 1.0 ng per reaction or Cp>32.0) will be analyzed as invalid.

3 Disclaimer

DNA-Technology Genotyping assays provide genetic information for some, but not all polymorphic loci known to be associated with certain medical conditions. This information estimates a probability of disease development but does not provide a definitive diagnosis, since other genes may contribute to the odds of disease onset. Moreover, the professional medical consultation regarding complex diseases cannot solely rely on genetic testing. The medical recommendations should also consider behavioral, physical, nutritional and familial information of a patient. On the basis of DNA-Technology Genotyping assays, a specialist can conclude whether a person of a certain genotype has lower or higher chance of disease development in relation to average risk. The definitive diagnosis is a derivative of a physicians experience and the depth of clinical information.

At the assay development stage we review the most up-to-date scientific literature on genetic associations repeatedly confirmed by independent research. We restrict our genotyping assays to a relatively small set of genetic markers because we believe they provide the most helpful and unbiased information about possible genetic susceptibility to common diseases.

Storage and handling requirements

The PCR-mix, PCR-buffer and mineral oil must be stored at temperatures between 2 °C and 8 °C and out of light during the storage period. The excessive temperature and light can be detrimental to product performance.

The Taq-AT polymerase must be stored at minus 20 °C during the storage period.

Transportation can be held by all types of roofed transport with adherence to above mentioned temperature requirements.

Expiration date – 6 months from the date of Quality Control Department approval in compliance with all transportation, storage and operation conditions.

Contact our customer service department regarding issues of quality of the “Pharmacogenetics” Real-time PCR based SNP genotyping assay:

Phone: +7 (800) 200-75-15,
Phone/Fax: +7(495) 640-17-71
E-mail: hotline@dna-technology.ru, www.dna-technology.ru
Address: 117587, Moscow, Varshavskoye sh., 125g, building 6, DNA Technology
## Table 1

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype</th>
<th>Fam,°C</th>
<th>Hex,°C</th>
<th>Genotype</th>
<th>Fam,°C</th>
<th>Hex,°C</th>
<th>Genotype</th>
<th>Fam,°C</th>
<th>Hex,°C</th>
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</thead>
<tbody>
<tr>
<td>CYP2C9: 430 C&gt;T (Arg144Cys)</td>
<td>CC</td>
<td>58.8</td>
<td>46.7</td>
<td>TT</td>
<td>51.0</td>
<td>56.0</td>
<td>CT</td>
<td>58.6</td>
<td>56.6</td>
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<tr>
<td>CYP2C9: A&gt;C (Ile359Leu)</td>
<td>AA</td>
<td>57.4</td>
<td>48.4</td>
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<td>59.0</td>
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<td>CYP4F2: C&gt;T (Val433Met)</td>
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<td>57.8</td>
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<tr>
<td>VKORC1: -1639 G&gt;A</td>
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<td>48.0</td>
<td>AA</td>
<td>40.5</td>
<td>54.7</td>
<td>GA</td>
<td>53.0</td>
<td>54.3</td>
</tr>
</tbody>
</table>

Appendix.

The DNA-Technology genetic tests are performed in certified laboratory. The tests have been analytically validated with regard to current standards in clinical laboratory practices.