Human CYP2C19 Genotype Detection Kit
(PCR-Fluorescence Probe)

[Product Name]
Human CYP2C19 Genotype Detection Kit (qPCR- Probe)

[Packing Specification] 24rxns/kit

[Intended Use]
This kit is used for the detection of Human CYP2C19 gene in blood and samples. It offers auxiliary means for the genotype of the CYP2C19*2(c.681G>A) and CYP2C19*3(C.636G>A), which support a reference for the individualized medical treat.

The major advantage of this kit is one-step, without nucleic acid extraction and purification. Directly add the blood in the PCR reaction solution, which shorten the testing time and reduce the cost.

Table 1: The gene and test fluorescence channel

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene polymorphism</th>
<th>Test channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19*2</td>
<td>CYP2C19*2G</td>
<td>FAM</td>
</tr>
<tr>
<td>CYP2C19*2</td>
<td>CYP2C19*2A</td>
<td>Texas Red</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>CYP2C19*3G</td>
<td>FAM</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>CYP2C19*3A</td>
<td>Texas Red</td>
</tr>
</tbody>
</table>

[Detection Principle]
This kit adopts PCR method combined with fluorescence probe in vitro amplification technology to test quantitatively the genesite of CYP2C19*2 and CYP2C19*3.

On the basis of their ability to metabolize, individuals can be classified as extensive metabolizers (EM), poor metabolizers (PM) or intermediate metabolizers (IM). The EM only carry the CYP2C19*1, the IM carry CYP2C19*2 or CYP2C19*3, and the PM carry include CYP2C19*2/*2, CYP2C19*2/*3 and CYP2C19*3/*3. In china CYP2C19*2 (rs4244285, c.681G>A) and CYP2C19*3 (rs4986893, c.636G>A) are the major alleles.

[Kit Contents]
Table 2: Kit Content

<table>
<thead>
<tr>
<th>No.</th>
<th>Content</th>
<th>24 rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CYP2C19*2 PCR reaction mix</td>
<td>1 vial, 1.3mL</td>
</tr>
<tr>
<td>2</td>
<td>CYP2C19*3 PCR reaction mix</td>
<td>1 vial, 1.3mL</td>
</tr>
<tr>
<td>3</td>
<td>CYP2C19*2 positive control</td>
<td>1 vial, 15μL</td>
</tr>
<tr>
<td>4</td>
<td>CYP2C19*3 positive control</td>
<td>1 vial, 15μL</td>
</tr>
<tr>
<td>5</td>
<td>Negative control</td>
<td>1 vial, 30μL</td>
</tr>
<tr>
<td>6</td>
<td>User Manual</td>
<td>1</td>
</tr>
</tbody>
</table>

PCR reaction mix: include the special primers and probes target to CYP2C19*2 and CYP2C19*3, qPCR master mix.

Note: Different batch contents in the kit shall not be interchangeable.

[Storage]
All reagents should be stored at below -20° C. Storage at +4° C is not recommended.

[Warnings and Precaution]
1. Sample type: Whole blood
2. Sample requirements
Venous blood: Extract 3 ml venous blood to the collection tubes, which contain appropriate anticoagulant in vitro (suggest sodium citrate anticoagulation tubes, can also use EDTA anticoagulant tubes, cannot use heparin anticoagulant tubes).

Finger blood: Clean the finger with medical alcohol disinfection at first. Use one-time use of straw to the end-of-life pain-free blood collection needles to get blood, then move the blood to the PCR tube contained sodium citrate anticoagulation.

The collected blood samples can be stored for a longer time when stores at low temperature.

[Applicable instrument]
Coyote Bio Mini8 Real-Time PCR System
ABI 7500
Roche 480

[Procedure]
1. Sample Preparation/Treatment
   This kit does not need sample nucleic acid extraction and purification.

   Whole blood collected in sodium citrate anticoagulant. Serum sample should be separated from the whole blood for the following detection.

2. Preparation of amplification reagent
   PCR reaction mix 49.5 μL + whole blood 0.5μL

3. Adding samples and controls to the reaction tubes
   Separately add the samples, positive control and blank control to different tubes:
   1) Separately add 0.5μL whole blood into the sample reaction tube.
   2) Separately add 0.5μL Positive control into the reactions as positive control.
   3) Separately add 0.5μL Negative control to the reaction as negative control.

   Close the tube, mix thoroughly and spin down the mixture.

   Operation should be performed on the ice-bath. 2000rpm spin for 10sec. Put the reaction tube on the test instrument.

1. PCR Amplification
   Perform the following protocol in the instrument:

Table 3: Instrument parameter settings

No. | Gene polymorphism | Test channel |
--- |-------------------|--------------|
1 | CYP2C19*2          | FAM          |
2 | CYP2C19*3          | Texas Red    |
Selection of fluorescence channels:
Reporter FAM: G allele
Reporter Texas Red: A allele
Please refer to the instrument manual for specific channel set detection.

[Quality control]
Negative control: Both the FAM detection channel and the Texas Red detection channel should not have obvious logarithmic growth period;
Positive control: Both detection channel should have the logarithmic growth period, and the Ct value \( \leq 25 \); Above condition should be all applied, this test is valid, or the test is invalid.

[Results interpretation]
a. if the sample Ct value \( \leq 28 \), report positive the right allele;
b. if the sample Ct value \( 30 > Ct > 28 \), please retest the sample. If the repeat result still \( > 28 \), and the negative control is no value, the result is the right allele, and if the repeat sample result is no value, report as negative.
c. if the sample Ct value is \( > 28 \) or no value, report negative for the allele.

Table 4: Results Interpretation

<table>
<thead>
<tr>
<th>Pro.</th>
<th>Tem.</th>
<th>Time</th>
<th>Cycle No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95°C</td>
<td>5s</td>
<td>15 cycles</td>
</tr>
<tr>
<td></td>
<td>50°C</td>
<td>10s</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>95°C</td>
<td>1min</td>
<td>1 cycle</td>
</tr>
<tr>
<td>3</td>
<td>95°C</td>
<td>5s</td>
<td>40 cycles</td>
</tr>
<tr>
<td></td>
<td>51°C</td>
<td>10s</td>
<td>(reading)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FAM channel</th>
<th>Texas Red channel</th>
<th>Results Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>GG</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>GA</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>AA</td>
</tr>
</tbody>
</table>

[Limitations of the assay]
1. Detection result of this kit is only for clinical reference, clinical diagnosis and treatment to patients should be considered other factors as symptoms, medical history, other laboratory tests and therapeutic reaction.
2. Aerosol pollution may cause the false positive easily, please pay attention to the processing of PCR products.

[Product performance index]
1. Limit of detection: The LOD of this kit is 50 copies/reaction;
2. The reference product coincidence: Test 4 mutation references and get the mutations; test 2 wild types and only get homozygote not mutation at all;
3. Precision: Test both high and low concentration of the GA reference for 10 times , and get the correct result, beside the Ct value’s CV \( \leq 5\% \).

[Warnings and Precaution]
1. For research only.
2. Do not use the kit after its expiration date;
3. Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use;
4. Avoid the cross use for pollution prevention;
5. Remove the PCR product to a hermetic bag to avoid the potential aerosol pollution;
6. Avoid the bubbles after subpackage the mixture, and cover the lid of PCR tubes carefully;
7. Infectious clinical samples need to be operated in the biosafety cabinet;
8. Please discard the used tips in the waste cylinders contained disinfection solution. Discard them with other waste after the high pressure sterilization.
9. Treat the laboratory tables and other tools with 1% sodium hypochlorite, 75% alcohol or ultraviolet light disinfection.

[Reference]