Table of Contents

Description ........................................................................................................................... 2

Kit Components .................................................................................................................. 2

Reagents not supplied in the kit .......................................................................................... 2

Equipment required ............................................................................................................ 2

Storage ................................................................................................................................. 2

References ............................................................................................................................ 2

Principle ............................................................................................................................... 3

Protocol ............................................................................................................................... 4

Identification of HPV types ................................................................................................ 5

Control Template ................................................................................................................. 6

Related productsV ............................................................................................................... 6
Description:

PCR Human Papillomavirus Typing Set (50 assays) Cat.#6603

PCR Human Papillomavirus Typing Set is a primers set designed to identify the type of a broad range of human papillomavirus (HPV) using the polymerase chain reaction (PCR). This set utilizes two pairs of consensus primers designed from the homologous region in HPVs to allow the common amplification of the sequence containing E6 and E7 regions of HPV (228–268 bp). Of the HPVs, malignant HPV-16, -18, -33, -52b and -58 are amplified using the HPvPpU-1M/HPvPpU-2R primers pair; benign HPV-6, and -11 are amplified using the HPvPpU-3B/HPvPpU-2R primers pair. Digestion of PCR products with restriction enzymes (Acc I, Afa I, Ava I, Ava II and Bgl II) and subsequent agarose gel electrophoresis allow the identification of HPV types. This primers set will work most efficiently when used in conjunction with TaKaRa Taq™ (TaKaRa Cat.#R00).

Kit Components:

( 50 reactions x 100 μl PCR or 100 reactions x 50 μl PCR)

1. HPvPpU-1M (malignant forward primer) (25 pmol/µl) 50 µl
2. HPvPpU-3B (benign forward primer) (25 pmol/µl) 50 µl
3. HPvPpU-2R (common reverse primer) (25 pmol/µl) 100 µl
4. Control Template HPV-TM (malignant) (1 ng/µl) 50 µl
   HPV-TB (benign) (1 ng/µl) 50 µl

Reagents not supplied in the kit:

1. TaKaRa Taq™ (TaKaRa Cat.#R001)
2. Agarose gel
   NuSieve® 3:1 Agarose (Cambrex)
3. Proteinase K
4. 10x PCR Buffer* 100 mM Tris-HCl buffer, pH8.3
   500 mM KCl
   15 mM MgCl₂
5. dNTP Mixture* (dATP, dCTP, dGTP and dTTP, ea. 2.5 mM)
   *Supplied with TaKaRa Taq™.

Equipment required:

1. Authorized instruments for PCR
   TaKaRa Thermal Cycler Dice (TaKaRa Cat.#TP600/TP650)
2. Microcentrifuge tubes (made of polypropylene)
3. Agarose gel electrophoresis apparatus
4. Microcentrifuge
5. Micropipets and pipette tips (autoclaved)

Storage:

-20°C

References:

Principle: Location and sequence of the primers (Table 1)

Table 1: Alignment of the consensus primers with the corresponding sequences of several HPV types.

<table>
<thead>
<tr>
<th></th>
<th>5’ Nucleotide Mismatches</th>
<th>Amplification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HPVpU-1M</strong></td>
<td><strong>5’-TGTCAAAAACCGTTGTCCTCC-3’</strong></td>
<td></td>
</tr>
<tr>
<td>HPV6</td>
<td>-----C-----C-----GA</td>
<td>420 4 ×</td>
</tr>
<tr>
<td>HPV11</td>
<td>-----C-----C-----GA</td>
<td>420 4 ×</td>
</tr>
<tr>
<td>HPV16</td>
<td>-----G-----AC-----</td>
<td>419 3 ○</td>
</tr>
<tr>
<td>HPV18</td>
<td>-----C-----G-----AA</td>
<td>426 4 ○</td>
</tr>
<tr>
<td>HPV31</td>
<td>-----G--------</td>
<td>423 1 ○</td>
</tr>
<tr>
<td>HPV33</td>
<td>-----G-----T------</td>
<td>424 2 ○</td>
</tr>
<tr>
<td>HPV35</td>
<td>-----C--------</td>
<td>425 1 ○</td>
</tr>
<tr>
<td>HPV52b</td>
<td>-----CG-----A----</td>
<td>418 4 ○</td>
</tr>
<tr>
<td>HPV58</td>
<td>-----G--------</td>
<td>425 2 ○</td>
</tr>
<tr>
<td><strong>HPVpU-31B</strong></td>
<td><strong>5’-TGCTAATTCCGTGCTACCTG-3’</strong></td>
<td></td>
</tr>
<tr>
<td>HPV6</td>
<td>-------------------</td>
<td>400 0 ○</td>
</tr>
<tr>
<td>HPV11</td>
<td>-T-----T-----T-----</td>
<td>400 3 ○</td>
</tr>
<tr>
<td>HPV16</td>
<td>-T-----A-----TATTAAC</td>
<td>399 9 ×</td>
</tr>
<tr>
<td>HPV18</td>
<td>-AT-----AA-----CTG-G-</td>
<td>406 8 ×</td>
</tr>
<tr>
<td>HPV31</td>
<td>-T-----A-----TATAAC</td>
<td>403 8 ×</td>
</tr>
<tr>
<td>HPV33</td>
<td>-AT-----A-----TATTA-A</td>
<td>404 9 ×</td>
</tr>
<tr>
<td>HPV35</td>
<td>-AT-----A-----TATTAACA</td>
<td>405 10 ×</td>
</tr>
<tr>
<td>HPV52b</td>
<td>-AACT-----A-A-----TATAATTAC</td>
<td>398 12 ×</td>
</tr>
<tr>
<td>HPV58</td>
<td>-AT-----A-A-------TATTA-T</td>
<td>405 10 ×</td>
</tr>
<tr>
<td><strong>HPVpU-2R</strong></td>
<td><strong>5’-GAGCTGTCGGCTTAATTGCTC-3’</strong></td>
<td></td>
</tr>
<tr>
<td>HPV6</td>
<td>------TAC------</td>
<td>627 3 ○</td>
</tr>
<tr>
<td>HPV11</td>
<td>------TTC------</td>
<td>627 3 ○</td>
</tr>
<tr>
<td>HPV16</td>
<td>------AT------</td>
<td>656 2 ○</td>
</tr>
<tr>
<td>HPV18</td>
<td>TCTGA----------</td>
<td>693 5 ○</td>
</tr>
<tr>
<td>HPV31</td>
<td>-----GG--------</td>
<td>654 2 ○</td>
</tr>
<tr>
<td>HPV33</td>
<td>-----A--------</td>
<td>667 1 ○</td>
</tr>
<tr>
<td>HPV35</td>
<td>-----A--AC------</td>
<td>656 3 ○</td>
</tr>
<tr>
<td>HPV52b</td>
<td>-----A--C-------</td>
<td>648 2 ○</td>
</tr>
<tr>
<td>HPV58</td>
<td>-----A--------</td>
<td>668 2 ○</td>
</tr>
</tbody>
</table>
Protocol:

[A: Preparation of genome DNA]
1. Add 300 µl of reaction mixture (10 mM Tris-HCl, pH8.0, 5 mM EDTA, 0.5% SDS, 0.2 mg/ml Proteinase K) in a tube containing tissue specimens.
2. Incubating at 37℃ for 12 hours.
3. Add 300 µl of phenol/chloroform solution to the sample treated with Proteinase K at step 2 and mix.
4. Centrifuge at 12,000 rpm for 10 min and transfer the aqueous layer (upper layer) into a fresh tube.
5. Add 300 µl of chloroform/isooamyl alcohol solution and mix.
6. Centrifuge at 12,000 rpm for 10 min and transfer the aqueous layer (upper layer) into a fresh tube.
7. Add 600 µl of ethanol and 30 µl of 3 M CH3COONa and leave at -20℃ for 1 hour (or -70℃, 30 min.).
8. Centrifuge at 12,000 rpm for 10 min. and discard the supernatant.
9. Rinse the precipitate with 80% ethanol, and then dry under the reduced pressure.
10. Dissolve the genome DNA in distilled sterilized water. (The concentration of 0.1 µg/µl is advisable.)

[B: PCR reaction]
1. Preparation of reaction mixture
Prepare the reaction mixture by combining the following reagents and TaKaRa Taq (Cat.#R001).
2. If necessary overlay 50 - 100 µl of mineral oil depending on an used thermal cycler. Perform PCR under the following condition.
94℃ 30 sec
55℃ 2 min 30 cycles
72℃ 30 sec
3. After PCR reaction, remove mineral oil.
4. Take 10 µl of PCR reactant and apply for agarose gel* electrophoresis to verify the amplified DNA fragments. When HPV DNA is present, 228~268 bp band of amplified DNA appears.
*Use 4% NuSieve ® 3:1 Agarose (Cambrex) for gel electrophoresis.
5. When the electrophoresis results shows the 228~268 bp of amplified DNA fragments, purify those DNA; extract the reactant with phenol/chloroform solution and extract with chloroform, and then precipitate with ethanol.
6. Dissolve the precipitated DNA in 10 µl of TE buffer. (The concentration of DNA should be approx. 0.1–0.2 µg/µl.)

Identification of HPV genotypes by restriction enzymes analysis of PCR products:

Restriction enzyme digestion of PCR products generated using PCR Papillomavirus Typing Set and subsequent agarose gel analysis can identify HPV genotypes of samples.

<A. Typing of malignant HPV>

1. When the presence of amplified DNA is confirmed at step IV-B-3 after PCR reaction using HPVpU-1M/HPVpU-2R primer pairs, perform typing of malignant HPV by restriction enzymes digestion.

   1) Prepare the reaction mixture by combining the followings.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplified DNA</td>
<td>1 µl (0.1–0.2 µg)</td>
</tr>
<tr>
<td>10X Restriction Enzyme Buffer</td>
<td>2 µl</td>
</tr>
<tr>
<td>Ava II</td>
<td>0.5 µl (1–6 units)</td>
</tr>
<tr>
<td>Distilled sterilized water</td>
<td>16.5 µl</td>
</tr>
<tr>
<td>Total</td>
<td>20 µl</td>
</tr>
</tbody>
</table>

2) Digest DNA with Ava II by incubating the mixture at 37℃ for 1 hour.

3) After the digestion, apply the reactant for agarose gel electrophoresis to analyze the digestion patterns. HPV-6, -8 and -33 are identified by the digestion with Ava II. (Table 2-a)

2. When the amplified DNA is not digested with Ava II, identify the genotypes of HPV by following the same procedure described at the above, V-A-, using Bgl II, Afa I, Acc I, or Ava I in place of Ava II. (Table 2-a)

<B. Typing of benign HPV>

When the presence of amplified DNA fragments are verified after PCR reaction using the HPVpU-3B/HPVpU-2R primer pair at IV-B-3, perform typing of benign HPV by digestion with Afa I. Follow the procedure described at A-1 to digest the amplified DNA fragments to distinguish between HPV-6 and HPV-11. (Table 2-b)

Table 2

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Total length (bp)</th>
<th>Restriction Enzyme</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPV16</td>
<td>HPV18</td>
<td>HPV31</td>
</tr>
<tr>
<td>a) HPVpU-1M/HPVpU-2R</td>
<td>238</td>
<td>268</td>
<td>232</td>
</tr>
<tr>
<td>Restriction Ava II</td>
<td>157/81</td>
<td>172/96</td>
<td>NC</td>
</tr>
<tr>
<td>Afa I</td>
<td>NC</td>
<td>NC</td>
<td>117/115</td>
</tr>
<tr>
<td>Bgl II</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Acc I</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Ava I</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>b) HPVpU-3B/HPVpU-2R</td>
<td>228</td>
<td>228</td>
<td>228</td>
</tr>
<tr>
<td>Restriction Afa I</td>
<td>132/96</td>
<td>166/62</td>
<td>NC</td>
</tr>
<tr>
<td>enzyme Ava I</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Bgl II</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Acc I</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Ava I</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

*The number shows the fragment length after restriction enzymes digestion.
NC, No cut.
Control Template:  This set includes both malignant and benign control template for verification of the PCR reaction by using the primer pairs provided in the set. 1 µl of supplied control template is used in one reaction. Approximately 60bp of PCR products are obtained with Control Template, and its size is different from the products derived from HPV. Restriction enzyme digestion of PCR products can also be confirmed because PCR products obtained using control template contains the sites of Ava II, Afa I, Bgl II, Acc I, and Ava I and small sizes of DNA fragments are yielded by digestion of restriction enzymes.

<table>
<thead>
<tr>
<th>Control template</th>
<th>HPV-TM</th>
<th>HPV-TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primers</td>
<td>pU-1M/pU-2R</td>
<td>pU-31B/pU-2R</td>
</tr>
<tr>
<td>Amplified length(bp)</td>
<td>63</td>
<td>61</td>
</tr>
</tbody>
</table>

Related products:  
- TaKaRa Taq™ (Cat.#R001)  
- PCR Human Papillomavirus Detection Set (Cat.#6602)  
- TaKaRa Thermal Cycler Dice (Cat.#TP600/650)

Note:  
This product is intended to be used for research purpose only. They are not to be used for drug or diagnostic purposes, nor are they intended for human use. They shall not to be used products as food, cosmetics, or utensils, etc. Takara products may not be resold or transfered, modified for resale or transfer, or used to manufacture commercial products without written approval from TAKARA BIO INC.  
If you require licenses for other use, please call at +81 77 543 7247 or contact from our website at www.takara-bio.com.