Cat. # 6603

For Research Use

TaKaRa

TaKaRa PCR Human Papillomavirus Typing Set

Product Manual

v202002Da

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I. Description

PCR Human Papillomavirus Typing Set is a primer set to identify the specific type of human papillomavirus (HPV) by 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, -31, -33, -35, -52b and -58 are amplified using the HPVpU-1M/HPVpU-2R primers pair, and benign HPV-6, and -11 are amplified using the HPVpU-31B/HPVpU-2R primers pair. Digestion of PCR products with restriction enzymes (*Acc* I, *Afa* I, *Bme*T110 I (*Ava* I), *Vpa*K11B I (*Ava* II) and *Bgl* II) and subsequent agarose gel electrophoresis allow the identification of HPV types. These primer sets will work most efficiently when used in conjunction with *TaKaRa Taq* (Cat. #R001).

This kit includes both malignant and benign control templates for verification of the PCR results by using the primer pairs provided in the kit. PCR products (approximately 60 bp) obtained using Control Template are smaller than products amplified from HPV PCR products from the Control Template can also be digested using the same restriction enzymes (*Acc* I, *Afa* I, *Bme* T110 I (*Ava* I), *Vpa* K11B I (*Ava* II) and *Bgl* II) yielding small DNA fragments.

Control Template	HPV-TM	HPV-TB
Primers	pU-1M/pU-2R	pU-31B/pU-2R
Amplified length (bp)	63	61

II. Components (100 reactions, 50 μl PCR)

1.	HPVpU-1M (malignant forward primer)	25 pmol/μl	50 μl
2.	HPVpU-31B (benign forward primer)	25 pmol/μl	50 μl
3.	HPVpU-2R (common reverse primer)	25 pmol/μl	100 μl
4.	Control Template HPV-TM (malignant)	1 ng/μl	50 μl
5.	Control Template HPV-TB (benign)	1 ng/μl	50 μl

III. Materials Required but not Provided

[Reagents]

1. *TaKaRa Taq* (Cat.#R001) (including 10X PCR Buffer, dNTP Mixture)
2. Enzyme Set A (Cat.#6604) (Restriction enzyme set)
3. Agarose
   PrimeGel™ Agarose PCR-Sieve (Cat.#5810A)
4. Proteinase K
5. Phenol/Chloroform/Isoamylalcohol
6. chloroform/isoamyl alcohol
7. Ethanol

[Materials]

1. TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat.#TP600) or
   TaKaRa PCR Thermal Cycler Dice *Touch* (Cat.#TP350)
2. Microcentrifuge tubes (made of polypropylene)
3. Agarose gel electrophoresis apparatus
4. Microcentrifuge
5. Micropipets and pipette tips (autoclaved)
6. NucleoSpin Gel and PCR Clean-up (Cat. #740609.10/50/250)

IV. Storage

-20℃

V. Principle

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

		5' Nucleotide	Mismatches	Amplification
HPVpU-1M	5'-TGTCAAAAACCGTTGTGTCC-3'			
HPV6	---------C----------C---------GA	420	4	×
HPV11	---------C----G----------GA	420	4	×
HPV16	----------G---AC----------	419	3	○
HPV18	--C------G----------AA------	426	4	○
HPV31	----------G----------	423	1	○
HPV33	----------G------T----------	424	2	○
HPV35	----------C----------	425	1	○
HPV52b	----------CG----A---A----------	418	4	○
HPV58	----------G------A----------	425	2	○
HPVpU-31B	5'-TGCTAATTCGGTGCTACCTG-3'			
HPV6	----------	400	0	○
HPV11	----T----------T-----T----------	400	3	○
HPV16	----T---------A---------TATTAAC	399	9	×
HPV18	--AT-------AA----------CTG--G-	406	8	×
HPV31	----T---------A---------TATAAC-	403	8	×
HPV33	---AT---------A---------TATTA-A	404	9	×
HPV35	---AT---------A---------TATTACA	405	10	×
HPV52b	---AACT-----A--A-----TATAA--T	398	12	×
HPV58	---AT---------A--A-----TATTA--T	405	10	×
HPVpU-2R	5'-GAGCTGTCGCTTAATTGCTC-3'			
HPV6	----------ATC----------	627	3	○
HPV11	----------TTC----------	627	3	○
HPV16	----------AT----------	656	2	○
HPV18	TCTGA----------	693	5	○
HPV31	----------GG----------	654	2	○
HPV33	----------A----------	667	1	○
HPV35	----------A---AC----------	656	3	○
HPV52b	----------A---C----------	648	2	○
HPV58	----------A---A----------	668	2	○

VI. Protocol

1. Preparation of genomic DNA

a. Prepare Proteinase K Reaction Mixture (10 mM Tris-HCl, pH 8.0, 5 mM EDTA, 0.5% SDS, 0.2 mg/ml Proteinase K) at 300 μl/sample.
b. Add 300 μl of the Proteinase K Reaction Mixture in a tube containing tissue specimens.
c. Incubate at 37℃ for 12 hours.
d. Add 300 μl of phenol/chloroform/isoamylalcohol and vortex well.
e. Centrifuge at 12,000 rpm for 10 min at room temperature and transfer the aqueous layer (upper layer) into a new tube.
f. Add 300 μl of chloroform/isoamyl alcohol and vortex well.
g. Centrifuge at 12,000 rpm for 10 min at room temperature and transfer the aqueous layer (upper layer) into a new tube.
h. Add 600 μl of ethanol and 30 μl of 3 M CH_3COONa and leave at -20℃ for 1 hour (or -70℃, 30 min).
i. Centrifuge at 12,000 rpm for 10 min at 4 ℃ and discard the supernatant.
j. Rinse the precipitate with 80% ethanol, and then dry it under the reduced pressure.
k. Dissolve the DNA in sterile purified water.

2. PCR

a. Preparation of reaction mixture
Prepare the reaction mixture by combining the following reagents and *TaKaRa Taq* (Cat. #R001).

<Amplification of malignant HPV DNA types>

Reagent	For 50 μl reaction
10X PCR Buffer	5 μl
dNTP Mixture (ea. 2.5 mM)	4 μl
HPVpU-1M	0.5 μl
HPVpU-2R	0.5 μl
TaKaRa Taq (5 U/μl)	0.25 μl
Sample genome DNA*	0.5 μg
Sterile purified water	up to 50 μl

<Amplification of benign HPV DNA types>

Reagent	For 50 μl reaction
10X PCR Buffer	5 μl
dNTP Mixture (ea. 2.5 mM)	4 μl
HPVpU-31B	0.5 μl
HPVpU-2R	0.5 μl
TaKaRa Taq (5 U/μl)	0.25 μl
Sample genome DNA*	0.5 μg
Sterile purified water	up to 50 μl

* 1 μl of the Control Template is used per reaction.

b. Perform PCR at the following condition.

94℃ 30 sec
55℃ 2 min ＊] 30 cycles
72℃ 30 sec

＊ When amplifying the same sample using both 31B/2R primers (benign) and 1M/2R primers (malignant), we recommend reducing the annealing time to 1 or 0.5 min. However, doing so may decrease the amplification efficiency.

c. Take 10 μl of PCR reactant and load on agarose gel for electrophoresis to verify the amplified DNA fragments. When HPV DNA is present, a 228-268 bp band appears.

Note: Use 4% gel of PrimeGel Agarose PCR-Sieve for gel electrophoresis.

d. Purify amplified DNA from the PCR reaction mixture by phenol/chloroform extraction or NucleoSpin Gel and PCR Clean-up.

f. Obtain the DNA solution (The recommended concentration of DNA is 0.1 - 0.2 μg/μl.)

VII. Identification of HPV genotypes by restriction enzymes analysis of PCR products

Restriction enzyme digestion of PCR products with PCR Human Papillomavirus Typing Set can identify HPV genotypes using Enzyme Set A.

Enzyme Set A (Cat. #6604)

1. Restriction Endonuclease
   *Vpa*K11B I (*Ava* II), *Afa* I, *Bgl* II, *Acc* I, *Bme*T110 I (*Ava* I) : each 10 U/ μl
2. 10X Universal Buffer K, M, H, T
   0.1% BSA
   10X *Vpa*K11B I Buffer

<Buffer Compositions>

10X K Buffer (For *Bme*T 110 I)
200 mM Tris-HCl (pH 8.5), 100 mM $MgCl_2$, 10 mM DTT, 1,000 mM KCl

10X M Buffer (For *Acc* I)
100 mM Tris-HCl (pH 7.5), 100 mM $MgCl_2$, 10 mM DTT, 500 mM NaCl

10X H Buffer (For *Bgl* II)
500 mM Tris-HCl (pH 7.5), 100 mM $MgCl_2$, 10 mM DTT, 1,000 mM NaCl

10X T Buffer (For *Afa* I)
330 mM Tris-acetate (pH 7.9), 100 mM Mg-acetate, 5 mM DTT, 660 mM K-acetate

0.1% BSA (For Afa I)

10X *Vpa*K11B I Buffer (For *Vpa*K11B I)
200 mM Tris-HCl (pH 7.5), 70 mM $MgCl_2$, 10 mM DTT, 2,000 mM KCl

A. Malignant HPV typing

1. When amplified DNA is present at PCR with HPVpU-1M/HPVpU-2R primer pairs (Section VI.2), perform typing of malignant HPV by restriction enzyme digestion.
   1) Prepare the reaction mixture by the following.

Reagent	Amount
Amplified DNA	1 μl (0.1 - 0.2 μg)
10X Restriction Enzyme Buffer	2 μl
*Vpa*K11B I (*Ava* II)	0.5 μl (5 U)
Sterile purified water	16.5 μl
Total	20 μl

   2) Incubate the mixture at 30℃ for 1 hour.
   3) After digestion, apply the reaction mixture on agarose gel electrophoresis to analyze the digestion patterns. HPV-16, -18 and -33 are identified by the digestion with *Vpa*K11B I (*Ava* II). (Table 2-a)
2. When the amplified DNA is not digested with *Vpa*K11B I (*Ava* II), identify the genotypes of HPV by following the same procedure described above using *Bgl* II, *Afa* I, *Acc* I, or *Bme*T110 I (*Ava* I) in place of *Vpa*K11B I (*Ava* II). Incubate at 37℃ for 1 hour. (Table 2-a)

B. Benign HPV typing

When amplified DNA is present at PCR with HPVpU-31B/HPVpU-2R primer pairs (Section VI.2), perform typing of benign HPV by digestion with *Afa* I. Follow the procedure described above to digest the amplified DNA fragments to distinguish between HPV-6 and HPV-11. (Table 2-b)

Table 2. Restriction fragment sizes of PCR products

a) HPVpU-1M/HPVpU-2R

HPV type	HPV 16	HPV 18	HPV 31	HPV 33	HPV 35	HPV 52b	HPV 58
Total length (bp)	238	268	232	244	232	231	244
*Vpa*K11B I (*Ava* II)	158/81	172/96	NC	136/108	NC	NC	NC
Afa I	NC	NC	117/118	NC	NC	NC	NC
Bgl II	NC	NC	NC	NC	NC	176/55	NC
Acc I	NC	NC	NC	NC	NC	NC	126/118
*Bme*T110 I (*Ava* I)	NC	NC	NC	NC	186/46	NC	NC

NC: No cut

b) HPVpU-31B/HPVpU-2R

HPV type	HPV 6	HPV 11
Total length (bp)	228	228
Afa I	132/96	166/62
*Vpa*K11B I (*Ava* II)	NC	NC
Bgl II	NC	NC
Acc I	NC	NC
*Bme*T110 I (*Ava* I)	NC	NC

VIII. References

Fujinaga Y, Shimada M, Okazawa K, Fukushima M, Kato I, and Fujinaga K. *Journal of General Virology*. (1991) **72**: 1039-1044.

IX. Related Products

TaKaRa Taq™ (Cat.#R001A/B/C)
Enzyme Set A (Cat. #6604)
PrimeGel™ Agarose PCR-Sieve（Cat.#5810A）
PCR Human Papillomavirus Detection Set (Cat. #6602)
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat.#TP600)
TaKaRa PCR Thermal Cycler Dice® *Touch* (Cat.#TP350)

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