Table of Contents

I. Description：

PCR Human Papillomavirus Detection Set is a primers set designed to detect the presence of human papillomavirus (HPV)16, 18, and 33 which have been found in cervical carcinoma at the high occurrence rates. This primers set allows simple and sensitive detection of HPV 16, 18, or 33 by specific amplification of their DNA using Polymerase Chain Reaction (PCR) technique. Included in this set are the specific primers for amplification of the sequence containing E6 region of HPV 16, 18, and 33 (140 bp for HPV 16, 18 and 141 bp for HPV 33) and the HPV type-specific probes for the subsequent hybridization. This primers set will work most efficiently when used in conjunction with *TaKaRa Taq* ™ (Cat.#R001).

II. Kit Components：

1.	HPVpF (common forward primer)		(25 pmol/μl)	100 μl
2.	HPVp16R (HPV 16 reverse primer)		(25 pmol/μl)	50 μl
3.	HPVp18R (HPV 18 reverse primer)		(25 pmol/μl)	50 μl
4.	HPVp33R (HPV 33 reverse primer)		(25 pmol/μl)	50 μl
5.	HPVb16 (HPV 16 probe)		(25 pmol/μl)	10 μl
6.	HPVb18 (HPV 18 probe)		(25 pmol/μl)	10 μl
7.	HPVb 33 (HPV 33 probe)		(25 pmol/μl)	10 μl
8.	Control Template HPV	T16(HPV16)	(1 ng/μl)	50 μl
		T18(HPV18)	(1 ng/ μ l)	50 μl
		T33(HPV33)	(1 ng/ μ l)	50 μl

III. Reagents not supplied in the kit：

1. *TaKaRa Taq* ™ (Cat.#R001)
2. DNA 5'end-labeling kit MEGALABEL ™ (Cat.#6070)
3. Agarose gel
   NuSieve® 3:1 Agarose (Lonza Rockland, Inc.)
4. Proteinase K (Cat.#9033)
5. 10x PCR Buffer ＊
   100 mM Tris-HCl buffer, pH8.3
   500 mM KCl
   15 mM $MgCl_2$
6. dNTP Mixture ＊ (ea. 2.5 mM)
7. [γ - ^{32}P] ATP (370 MBq/ml)

＊：Supplied with *TaKaRa Taq* ™

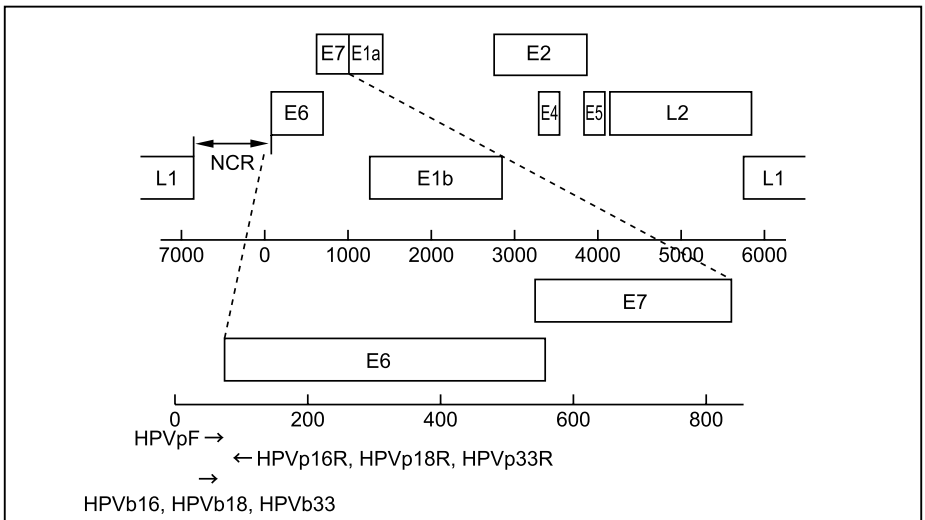
IV. Equipment required：

1. Authorized Thermal Cycler
   TaKaRa PCR Thermal Cycler Dice ™ (Gradient Model) (Cat.#TP600)
   TaKaRa PCR Thermal Cycler Dice ™ (Standard Model) (Cat.#TP650)
   TaKaRa PCR Thermal Cycler Dice ™ mini (Cat.#TP100)
2. Microcentrifuge tubes (made of polypropylene)
3. Agarose gel electrophoresis apparatus
4. Microcentrifuge
5. Micropipets and pipette tips (autoclaved)

V. Storage： －20 ℃

VI. Principle：

Fig 1. Amplified region and primer sequences



HPVpF	5'-AAGGGCGTAACCGAAATCGGT-3'
HPV16R	5'-GTTTGCAGCTCTGTGCATA-3'
HPV18R	5'-GTGTTCAGTTCCGTGCACA-3'
HPV33R	5'-GTCTCCAATGCTTGGCACA-3'
HPVb16	5'-CATTTTATGCACCAAAAGAGAACTGCAATG-3'
HPVb18	5'-TGAGAAACACACCACAATACTATGGCGCGC-3'
HPVb33	5'-CATTTTGCAGTAAGGTACTGCACGACTATG-3'

VII. 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. Incubate at 37 ℃ for 12 hours.
3. Add 300 μl of phenol/chloroform solution 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/isoamylalcohol 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 CH_3COONa 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.
10. Dissolve the genome DNA in sterile purified 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).

＜ CASE 1：Simultaneous amplification of HPV16, 18, and 33 DNA in a single tube ＞

For 100 μl reaction	
10 × PCR Buffer *	10 μl
dNTP Mixture (ea. 2.5 mM) *	8 μl
HPVpF	2 μl
HPVp16R	1 μl
HPVp18R	1 μl
HPVp33R	1 μl
TaKaRa Taq ™ (5 units/ μl)	0.5 μl
Sample genome DNA	1 μg
Sterile purified water	up to 100 μl

＊：Supplied with *TaKaRa Taq* ™

For 50 μl reaction	
10 × PCR Buffer *	5 μl
dNTP Mixture (ea. 2.5 mM) *	4 μl
HPVpF	1 μl
HPVp16R	0.5 μl
HPVp18R	0.5 μl
HPVp33R	0.5 μl
TaKaRa Taq ™ (5 units/ μl)	0.25 μl
Sample genome DNA	0.5 μg
Sterile purified water	up to 50 μl

＊：Supplied with *TaKaRa Taq* ™

＜ CASE 2：Amplification of HPV16, 18, and 33 DNA in each tube respectively ＞

For 100 μl reaction	
10 × PCR Buffer *	10 μl
dNTP Mixture (ea. 2.5 mM) *	8 μl
HPVpF	1 μl
HPVp16R or HPVp18R or HPVp33R	1 μl
TaKaRa Taq ™ (5 units/ μl)	0.5 μl
Sample genome DNA	1 μg
Sterile purified water	up to 100 μl

＊：Supplied with *TaKaRa Taq* ™

For 50 μl reaction	
10 × PCR Buffer *	5 μl
dNTP Mixture (ea. 2.5 mM) *	4 μl
HPVpF	0.5 μl
HPVp16R or HPVp18R or HPVp33R	0.5 μl
TaKaRa Taq ™ (5 units/ μl)	0.25 μl
Sample genome DNA	0.5 μg
Sterile purified water	up to 50 μl

＊：Supplied with *TaKaRa Taq* ™

2. If necessary, overlay 50-100 μl of mineral oil and perform PCR under the following condition.

94 ℃	30 sec	┐
55 ℃	2 min	] 30 cycles
72 ℃	30 sec	┘

3. Take 10 μl of PCR reactant and apply on agarose gel * electrophoresis to verify the amplified DNA. When target DNA is present, 140 bp of the amplified DNA appears. (When HPV 33 DNA is present, 141 bp of band appears.)
   ＊：4 % NuSieve® 3:1 Agarose (Lonza) is recommended for gel electrophoresis.

［C：Detection of HPV DNA by dot hybridization］

After simultaneous amplification of HPV 16, 18, and 33 DNA in a single tube, HPV DNA can be detected with high sensitivity by dot hybridization utilizing type-specific oligonucleotide probes. Oligonucleotides probes can be efficiently end-labeled with ^{32}P using DNA 5'end-labeling kit MEGALABEL ™ (Cat.#6070).

1. Fixation of amplified DNA onto a nylon membrane
   1) Transfer PCR reactant obtained at B into a fresh tube (0.5 ml) with special precautions to prevent carryover of mineral oil.
   2) Heat to denature the amplified DNA at 94 ℃ for 10 min.
   3) Leave the tube on ice for 5 min.
   4) Blot 1 μl of aliquot of PCR reactant onto each of three nylon membranes.
   5) Dry membranes and irradiate UV ray for 5 min to fix amplified DNA.

2. Prehybridization
   Put the membrane prepared at 1 respectively in hybridization packs containing prehybridization buffer ＊1 and perform prehybridization at 37 ℃ for 2 hours.

   ＊1：Prehybridization buffer
   - 5x Denhardts ＊2
   - 5x SSC
   - 0.1 % SDS
   - 0.1 mg/ml salmon sperm DNA

   ＊2：50x Denhardts stock solution
   - 1 % (w/v) Ficoll
   - 1 % Polyvinylpyrolidone
   - 1 % BSA

3. Probe labeling
   Label oligonucleotide probes with [γ-^{32}P] ATP using DNA 5'end-labeling kit MEGALABEL ™ (Cat.#6070). Please refer to the manual of MEGALABEL™ for the protocol ＊ (IV. Labeling of synthetic DNA).

   ＊1：Protocol：
   1) Combine the following reagents in a microcentrifuge tube.

HPVb16, HPVb18 or HPVb33	1 μl (25 pmol)
10x phosphorylation buffer	1 μl
T4 Polynucleotide Kinase	1 μl (10 units)
H_2O	2 μl
[γ-^{32}P] ATP (370 Mbq/ml) ＊2	5 μl

   ＊2：[γ-^{32}P] ATP is not provided in the MEGALABEL™.

   2) Incubate at 37 ℃ for 30 min.
   3) Inactivate the enzyme by heating the mixture at 70 ℃ for 5 ～ 10 min.

4. Hybridization
   1) Add each of the labeled probes (approx. 1×10^8 cpm) into the packs containing the prehybridization solution at 2.
   2) Incubate at 37 ℃ for 2 hours.

5. Wash the membranes twice in 2x SSC containing 0.1 % SDS for 10 min at room temperature, and then wash twice in 0.2x SSC containing 0.1 % SDS for 20 min at 55℃ .

6. Dry the membranes and perform autoradiography.

[D：Control Template]

This set includes Control Template to verify the PCR amplified DNA fragments. Amplification using HPVT16, HPVT18, HPVT33 as template and primer pairs of HPVpF/HPVp16R, HPVpF/HPVp18R or HPVpF/HPVp33R respectively yields 70 bp of DNA fragments. As this Control Template contains the sequences complimentary to the probes provided in this set, it can be used as positive control for each type of HPV in dot hybridization.

VIII. Reference：

Shimada, M.,Fukushima, M., Mukai, H., Kato, I., Nishikawa, A. and Fujinaga, K. (1990) *Jpn. J. Cancer Res*. **81**, 1-5.

IX. Related Products：

TaKaRa Taq ™（Cat.#R001A/B/C）
MEGALABEL ™（Cat.#6070）
Proteinase K（Cat.#9033）

URL:http://www.takara-bio.com
Phone: +81-77-543-7247 Fax: +81-77-543-9254