

## Protocol Tip

### 1 Tips for isolation of viral nucleic acids

- In case of Liquid based cervical cytology specimen or Cervical swab specimen,

1) Centrifuge 1ml of specimen at 13,000 rpm at 15 mins

2) Pellets are directly put into a 1.5ml microcentrifuge tube and vortexed with 200ul of PBS. Follow the step of isolation kit manual

- Do not add Qiagen protease directly to Buffer AL.
- Before using for the first time, add the appropriate amount of ethanol (96-100%) to Buffer AW1 as indicated on the bottle.
- Before using for the first time, add the appropriate amount of ethanol (96-100%) to Buffer AW2 concentrate as indicated on the bottle.
- Direct use of isolated DNA is required to store at 4°C and for long-term storage of DNA, placing at -20°C is recommended.

### 2 Tips for PCR step

- It is important to melt the reagents (if stored at -20°C) completely and then centrifuge before use.
- Please be careful not to cross contaminate the PCR mastermix and samples from positive control.
- Repeated freeze-thaw cycles of 2x Multiplex Master Mix should be avoided since they may lead to decreased assay performance. Therefore, it is recommended to keep it at 4°C during use.

## Virus DNA

### 1 Isolation of viral nucleic acids (Equilibrate samples to room temperature)

200µl Specimen  
20µl QIAGEN Protease  
200µl Buffer AL

Pulse-vortexing for 15 s and then incubate 56°C for 10 mins.

Add 200µl of ethanol

Pulse-vortexing for 15 s

Apply all of the lysate onto the column and then centrifuge (8000 rpm, 1 min)

Place the column in a clean 2ml collection tube

Add 500µl of buffer AW1 and then centrifuge (8000rpm, 1 min)

Place the column in a clean 2ml collection tube

Add 500µl of buffer AW2 and then centrifuge (14,000rpm, 3mins)

Place the column in a clean 2ml collection tube

Centrifuge at full speed (14,000 rpm, 1 min) to dry the membrane completely

Place the column in a clean 1.5ml microcentrifuge tubes

Apply 100µl of Buffer AE to the center of the membrane

Incubate at room temperature for 2 mins

Centrifuge (8,000 rpm, 1 min)

## User Quick Protocol

### 2 Multiplex PCR step

- Add the following reagent to a PCR tube. Set up all reactions on Ice.

DNA	3µl
5X HPV4A ACE PM	4µl
8-Mop solution	3µl
2X Multiplex Master Mix	10µl
<b>Total volume</b>	<b>20µl</b>

#### Note

- 2x Multiplex Master Mix contains polymerase, so we recommend you to add it at the last step
- Mix all reagents gently by pipetting
- Briefly tapping or pipetting to remove the bubbles in the tube
- To perform the test with a large number of samples, it is recommended to prepare mixture before use

Mixture for different No. of specimen (Unit: µl)

No. of specimen	2	3	4	5	6	7	8	9	10
5X HPV4A ACE PM	8	12	16	20	24	28	32	36	40
8-Mop solution	6	9	12	15	18	21	24	27	30
2X Multiplex Master Mix	20	30	40	50	60	70	80	90	100

Note: Prepare a volume of mixture greater than that required for the total number of reactions to be performed

- Immediately commence the PCR using the following program

Segment	No. of cycle	Temperature	Duration
1	1	94°C	15 min
2	40	94°C	0.5 min
		60°C	1.5 min
		72°C	1.5 min
3	1	72°C	10 min

- Electrophoresis 2µl of the PCR products on Screentape system, 3µl of the PCR products on MultiNA system, or 5µl of the PCR products on a 2% agarose gel containing EtBr

## Amplicon Size Information

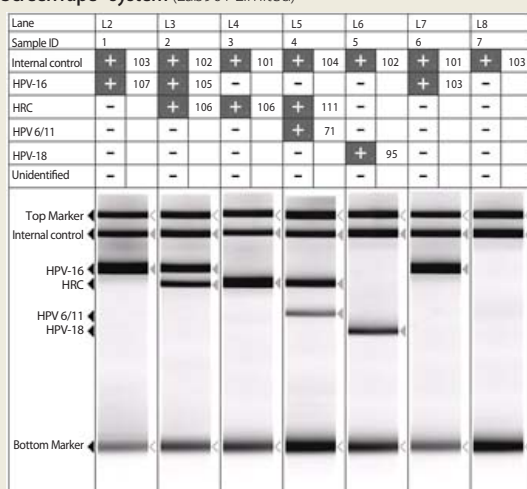
Table 1. HPV4A ACE Screening amplicon size

HPV4A ACE Screening	Size (bp)
	Agarose gel
Internal control	1000
HPV-16	588
HPV High Risk Common (HRC)	465
HPV-6/11	302
HPV-18	230

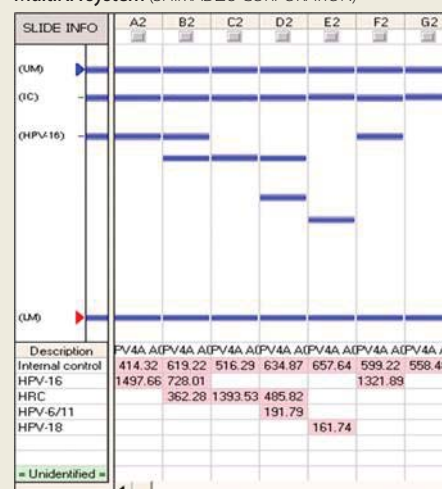
\* HRC (16 high-risk HPV types) : 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82  
High-risk HPV common primers might detect high-risk HPV types 39, 52 and 68 by cross-reactivity.

## Application to clinical samples

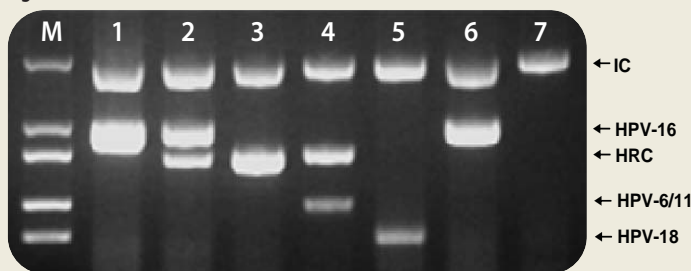
ScreenTape<sup>®</sup> system (Lab901 Limited)



MultiNA system (SHIMADZU CORPORATION)



Agarose Gel



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## Ordering information

Cat. No.	Product	Size
<b>Seeplex<sup>®</sup> HPV4A ACE Screening</b>		
HP6401Y	Seeplex <sup>®</sup> HPV4A ACE Screening	50 rxns
<b>ScreenTape<sup>®</sup> System</b>		
ST007	TapeStation	EA
ST105	DS12 ScreenTape (16 lane tape)	112T
ST214	ScreenTape Loading tips (16 lane tape)	Pack (3,840 tips)