GeneFinder™
" SMART Solution for Better Life"

ISO 13485 Certified

HPV-HR Real*Amp* Kit

REF **IFMR-26.02B100**

100 Tests / Kit

96 Tests / Kit (Only InGenius)

Store at -20℃ or below.
Shelf life is 12 months after manufacturing.

CE IVD ***Professional Use Only***

For ABI7500, ABI7500 Fast, CFX96, InGenius, Gentier 96E

INTENTED USE

GeneFinder™ HPV-HR Real*Amp* Kit is designed for screening and identification of 14 species of high risk HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68).
DNA extracted from cervical specimens by using Real-time PCR technology.

KIT COMPONENT

HPV-HR Real*Amp* Kit	100 Tests / Kit
HPV-HR Reaction Mixture*	1 X 1,300 µL
HPV-HR Probe Mixture**	1 X 780 µL
HPV-HR Positive control***	1 X 50 µL
HPV-HR Negative control****	1 X 50 µL

*, HPV-HR Reaction Mixture; *Taq* polymerase, $MgCl_2$, Buffer containing dNTPs
**, HPV-HR Probe Mixture; Oligonucleotides for amplification and detection of target
***, HPV-HR Positive Control; Clones for targets
****, HPV-HR Negative Control; Ultrapure quality water, PCR-grade

DESCRIPTION

- Human papillomavirus (HPV) is a DNA virus from the papillomavirus family that is capable of infecting humans. In particular, HPV16 and HPV18 are known to cause around 70% of cervical cancer cases. High-risk HPV infection is a cause of nearly all cases of cervical cancer.
- In real-time PCR, the amplified product is detected via fluorescent dye. The method relies on a DNA-based probe with a fluorescent reporter at one end, and a quencher of fluorescence at the opposite end of the probe.

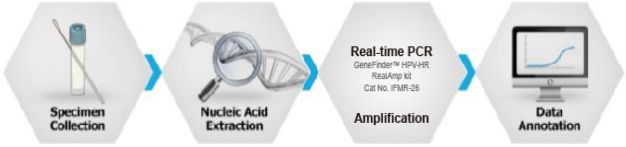
PREREQUISITE

- Applied Biosystems 7500 (ABI), Applied Biosystems 7500 Fast (ABF), CFX96 (CFX), InGenius Instrument System, Gentier 96E.
- Pipettes and pipettes tips with aerosol barrier.
- Vortex mixer.
- Centrifuge with rotor for microtiter plates.
- Disposable powder-free gloves.

WARNING AND PRECAUTION

- This product is designed for *In-vitro diagnostics* use.
- Do not use a kit after expiry date.
- The test has to be performed by well-trained and qualified personnel.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
- Unnecessary repeated freezing and thawing will be occurred inaccurate results.
- Do not mix reagent from different batches of the kit.
- Do not modify the reagent/sample volume used in the test or use in a wrong way which is not recommended.

WORK FLOW



PROTOCOL

A. Specimen

This product is must be used with genomic DNA samples extracted from cervical specimens.

B. DNA Extraction

It is recommended to use commercialized extraction product such as QIAamp DNA mini kit should be used.

C. Reagent Preparation

Before setting up PCR, all components need to be thawed, gently mixed and centrifuged briefly to collect solution at the bottom.

1. Mix 12.5 µL of HPV-HR Reaction Mixture, 7.5 µL of HPV-HR Probe Mixture to prepare master mixture per each reaction (refer to the below). Prepare enough volume of master mixture for all reactions plus extra to prevent pipetting error.
2. After mixing well, place 20 µL of master mixture into PCR tube or 96-well plate.
3. Add 5 µL of extracted DNA sample into tube or 96-well plate then mix all components by pipetting. Proceed in the same way with other DNA samples, positive and negative control (DNase/RNase free water).
4. Accurately close the tube with the cap or seal the 96-well plate.
5. Transfer the tubes or 96-well plate for test into the Real-time PCR and start for the amplification.

Component	Per reaction (µL)
HPV-HR Reaction Mixture	12.5
HPV-HR Probe Mixture	7.5
DNA sample or PC[1] or NC[2]	5
Total volume	25

[1], PC, positive control; [2], NC, negative control

D. Setting of Real-time PCR

This product is validated on For ABI7500 / ABI7500 FAST / CFX96 / InGenius / Gentier 96E.

1. Referring to the instrument manual, set on the dedicated software the parameters of thermal cycle.
2. Set up the PCR program and fluorescence as following, and then click the start "Run" button.

PCR program			
Cycle		Temp.	Time
Segment 1	1 cycle	50 °C	2 min
Segment 2	1 cycle	95 °C	10 min
Segment 3	45 cycle	95 °C	15 sec
		54 °C**	60 sec
		72 °C*	30 sec

*, Select 'Add plate Read to Step' in Extension stage (72 °C) at Step 3
**, InGenius Instrument Fluorescence acquisition set during annealing (54°C). It is set automatically.

(continued on reverse page)

Fluorescence setting			
Target	**ABI7500 / ABI7500 Fast Gentier 96E**	**CFX96**	***InGenius**
HPV Type #16	FAM	FAM	FAM
HPV Type #18	JOE	HEX	JOE/HEX
HPV High Risk (HR)	Texas Red	Cal fluor red 610	Texas red/Cal fluor red 610
IC	Cy5	Quasar 670	Cy5/Quasar 670

* InGenius instrument is set automatically.

E. Analysis Setting

Prior to analysis, set the threshold as below.

	Threshold setting		Baseline	
Target	**ABI7500/ ABI7500 Fast**	**CFX96/ Gentier 96E**	**Start**	**End**
HPV Type #16	20,000	300	3	15
HPV Type #18	20,000	300	3	15
HPV High Risk (HR)	20,000	300	10	15
IC	10,000	100	Auto	

* InGenius instrument is set automatically.

F. Result Interpretation

Here are examples for result interpretation if the sample is positive / negative.

Ct value				Positive / Negative				Assay Result
HPV #16	HPV #18	High Risk	I.C	HPV #16	HPV #18	High Risk	I.C	
≤45	≤45	≤45	≤35	+	+	+	+	HPV #16, #18, HR Positive
≤45	≤45	≤45	U.D or N/A or -*	+	+	+	-	HPV #16, #18, HR Positive
≤45	≤45	U.D or N/A or -	≤35	+	+	-	+	HPV #16, #18 Positive
≤45	≤45	U.D or N/A or -	U.D or N/A or -	+	+	-	-	HPV #16, #18 Positive
≤45	U.D or N/A or -	≤45	≤35	+	-	+	+	HPV #16, HR Positive
≤45	U.D or N/A or -	≤45	U.D or N/A or -	+	-	+	-	HPV #16, HR Positive
≤45	U.D or N/A or -	U.D or N/A or -	≤35	+	-	-	+	HPV #16 Positive
≤45	U.D or N/A or - **	U.D or N/A or -	U.D or N/A or -	+	-	-	-	HPV #16 Positive
U.D or N/A or -	≤45	≤45	≤35	-	+	+	+	HPV #18, HR Positive
U.D or N/A or -	≤45	≤45	U.D or N/A or -	-	+	+	-	HPV #18, HR Positive
U.D or N/A or -	≤45	U.D or N/A or -	≤35	-	+	-	+	HPV #18 Positive
U.D or N/A or -	≤45	U.D or N/A or -	U.D or N/A or -	-	+	-	-	HPV #18 Positive
U.D or N/A or -	U.D or N/A or -	≤45	≤35	-	-	+	+	HPV HR Positive
U.D or N/A or -	U.D or N/A or -	≤45	U.D or N/A or -	-	-	+	-	HPV HR Positive
U.D or N/A or -	U.D or N/A or -	U.D or N/A or -	≤39	-	-	-	+	Negative
U.D or N/A or -	U.D or N/A or -	U.D or N/A or -	U.D or N/A or -	-	-	-	-	Invalid

*, U.D: Undetermined (or N/A: Not applicable or '-')

QUALITY CONTROL

- Positive Control and negative Ct range should be as below;

Acceptable Range of Positive Control (Ct value)			
HPV 16	**HPV 18**	**HR (High Risk)**	**IC**
22 ± 3	23 ± 3	22 ± 3	U.D or N/A

PERFORMANCE

Criteria	Results
Analytical Specificity	• 94 DNA/RNA samples were tested on the GeneFinder™ HPV-HR Real*AMP* Kit in order to evaluate the possibility of cross-reactivity. 94 DNA/RNA samples which have no concern with the detection target of the kit were negative * Cross-reactivity : 100% Specificity
Analytical Sensitivity	• Serial dilution (3,000, 300, 50, 10, 5, 1 copies/Test) of HPV-HR DNA (3 batches, 24 times of repeat test each) were tested * Analytical Sensitivity : 10 copies
Reproducibility	• Reproducibility was confirmed with identical standard substances at different conditions; different LOT, place, time and testers by 3 batch testing. * Criteria of safety was CV < 5% Value.
Freezing / Thaw Safety	• Freeze/Thaw safety of GeneFinder™ HPV-HR Real*AMP* Kit was confirmed 16 times by Freeze/Thaw repeat test. * Criteria of safety was CV < 5% of Ct Value

TROUBLE SHOOTING

Problem	Possible Cause	Recommendation
No signal in all samples including positive control	Error in master mixture preparation	Check the dispensing volume during preparation of master mixture
	Inhibitors added	Repeat the extraction step wit h new sample
	Probe degradation	Use a new probe reagent
	Positive control degradation	Use a new positive control
	Omitted components	Verify each component, repeat the PCR mixture Preparation
	Instrument setting error	Check the position setting for the positive control on the instruments. Check the thermal cycle set tings on instrument
Signal in negative control	Carry-over contamination	Take care when dealing with the sample, negative control and positive control.
	Dispensing error on the Microplate (from ABI7500, ABI7500 FAST, CFX96, Gentier 96E).	Avoid spilling the contents of the sample test tube. Always change tips between one sample and another. Take care when dispensing samples, negative controls, and positive controls onto the tubes.
	Tube cap / sealing film error (from ABI7500, ABI7500 FAST ,CFX96, Gentier 96E)	Check the condition of closure for cap or sealing a film
	Contamination of the amplification mix	Use a new aliquot of amplific ation mix
	Contamination of extraction or amplification area	Clean surfaces and instruments with aqueous detergents, wash lab coats replace test tubes and tips in use.
Weak or no fluorescent signal in samples only	Poor DNA quality	Use recommended kit for DNA extraction and store extracted DNA at -20℃
	Insufficient volume of DNA	Repeat PCR reaction with correct volume of DNA
Strong or odd fluorescent signal in samples only (i.e. 30103 error from InGenius)	Too high concentration of target in the sample	Perform a 1:10 dilution of DNA and retest it
Weak or no fluorescent signal in positive control only	Positive control degradation	Use a new positive control
Diverse intensity of fluorescent signals	Pipetting error	Make sure that the equal volume of reactants is added in each tube or plate
	Contamination in the outer surface of PCR tubes, plate , or PCR Cassette	Wear gloves during the experiment

• Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established;

Manufactured by **OSANG Healthcare Co., Ltd.** 132, Anyangcheondong-ro, Dongan-gu, Anyang-si, Gyeonggi-do, 14040, Korea
For technical support, Tel. +82-31-460-9914 Fax. +82-31-460-9933 Web: http://www.osanghc.com

OHC
OSANG HEALTHCARE