



HPV Total & High Risk Master Mix
REF: CL07HRM-I

Detection of Papilloma virus (HPV) and High Risk strains by amplification of L1 and E6/E7 regions

INTRODUCTION AND PURPOSE OF USE

The HPV Total & High Risk system is a qualitative test that allows the DNA amplification, by means of PCR, of HPV total and High Risk strains. The amplification products (450 bp of L1 region and 250 bp or 268 bp of E6/E7 region) and is detectable by agarose gel electrophoresis.

CONTENT

The system contains 48 vials with 45 µl each of PCR master mix, sufficient to perform 48 amplification tests.

- RI:** HPV High Risk master mix
Upstream primer, downstream primer, dNTPs, Tris-HCl, KCl, MgCl₂, Taq Polymerase, Nuclease-free water

MATERIALS AND STRUMENTATION REQUIRED BUT NOT SUPPLIED

- Disposable latex powder-free gloves or similar material;
- Bench microcentrifuge (12,000 - 14,000 rpm);
- Micropipettes and Sterile tips with aerosol filter;
- Thermal cycler;
- Agarose gel;
- Horizontal electrophoresis unit and power supply for electrophoresis;
- U.V. transilluminator and camera for gel recording.

ACCESSORY PRODUCTS

EX05 – DNA extraction from blood or vaginal, cervical and urethral swab
The kit allows the DNA extraction from Human samples. The kit contains enough reagents to perform the DNA extraction from 50 samples.

CL54M - β-Globin

Control system of DNA extraction through the genomic amplification of β-Globin human gene.

CL07HRC

Positive control consists of plasmid DNA containing the genomic region of interest of the Human Papilloma virus (HPV) and quantified by spectrophotometric analysis (pHPV16).

SAMPLES AND STORAGE

The HPV Total & High Risk system must be used with extracted DNA from the following biological samples: Vaginal Swab or Cytobrush. The samples collected must be shipped and stored at +2/+8°C and utilized within 3 days from the collected data. Store the sample at -20°C if utilized after 3 days.

PRECAUTIONS USE

This master mix is for in vitro diagnostic (IVD), for professional use only and not for in vivo use. Keep the PCR Master Mix on ice while in use and return to -20°C immediately after the use.

At all times follow Good Laboratory Practice (GLP) guidelines.

Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.

Avoid any contact between hands and eyes or nose during specimens collection and testing.

Handle and dispose of all used materials into appropriate bio-hazard waste containers, It should be discarded according to local law.

Keep separated the extraction, the reagents preparation and products detection area.

Never pipette solutions by mouth
Amplification products must be handled in such a way to reduce dispersion into the environments much as possible, in order to avoid the possibility of contamination.

Avoid the air bubbles during the master mix dispensing. Eliminate them before starting amplification.

Wash hands carefully after handling samples and reagents
Do not mix reagents from different lots

It is not infectious and hazardous for the health (see Material Safety data Sheet – MSDS).

Do not eat, drink or smoke in the area where specimens and kit reagents are handled.

Read carefully the instructions notice before using this test. Do not use beyond the expiration date which appears on the package label.

Do not use a test from a damaged protective wrapper.

LIMIT OF THE METHOD

The extreme sensitivity of gene amplification may cause false positive due to cross-contamination between samples and controls. Therefore, you should:

- physically separate all the products and reagents used for amplification reactions from those used for other reactions, as well as from post-amplification products,
- use tips with filters to prevent cross-contamination between samples,
- use disposable gloves and often change them,
- carefully open test tubes to prevent aerosol formation,
- close every test tube before opening another one.

The proper functioning of the amplification mix depends on the correct collection, correct transportation, correct storage and correct preparation of a biological sample.

As with any diagnostic device, the results obtained with this product must be interpreted in consideration of all the clinical data and other laboratory tests done on the patient.

As with any diagnostic device, there is a residual risk of obtaining invalid results, false positives and false negatives with this product.

STORAGE AND STABILITY

Store the master mix at -20°. An intact and well stored product has a stability of 9 months from the date of production. Do not use beyond the expiration date which appears on the package label.

ANALITICAL PROCEDURE

PCR:

Amplification:

- prepare a number of PCR test tubes corresponding to the number of samples to be tested.
- add 5 µl of DNA extracted.
- put the test tubes in the thermal cycler and run the following programme:

cycles	denaturation	annealing	extension
1	95° C 5 min		
40	95° C 30 sec	50° C 30 sec	72° C 1 min
			72° C 7 min

The method is validated on thermal cyclers **Applied Biosystem** mod. 2700 and **Veriti, Eppendorf, Techne** mod. Progene, **Biorad** Genecycler.

INTERPRETATION OF RESULTS

The use of positive and negative control in each amplification session allows to verify the correct functioning of the amplification mix and the absence of any contamination.

Make sure that the negative control does not show a band identical to that appearing in the positive control to exclude any possibility of contamination or a false positive. The session is invalid and must be repeated from the amplification phase.

Ensure the correct amplification of the positive control represented by the two specific bands presence:

- band 450 bp: *Screening L1 region*
- band 250/268 bp: *High Risk E6/E7 region*

If the specific amplification product is absent in the positive control reaction, this means that problems have occurred during the amplification phase (inefficient or absent amplification) which may cause false negatives. The session is invalid and must be repeated from the amplification phase.

If a 450 bp band and a 250 bp or 268 bp band appear in your samples, they are positive for HPV High Risk (HPV 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 68). You can directly compare the band with that appearing in the positive control.

If a 450 bp band appears in your samples, they are positive for HPV Low Risk. You can directly compare the band with that appearing in the positive control.

Low molecular weight bands, which can appear in all samples, are produced by unincorporated primers. Such are not significant for the interpretation of results.

PERFORMANCES

Analytical sensitivity:

For the purposes of this evaluation is considered as analytical sensitivity the highest serum dilution (title) to which a positive sample can be subjected without the system losing the ability to detect it as positive. The analytical sensitivity of the system was assessed by analyzing plasmid DNA of most important HPV species (pHPV16 and

pHPV18), quantified by spectrophotometric analysis, containing the genomic regions of interest in serial dilutions from 3,000 copies to 30 copies/reaction.

Concentration	30 copies	300 copies	3000 copies
N° of Test	9	9	9
N° of Positive	0	9	9
N° of Negative	9	0	0

The Analytical sensitivity ≤ 300 copies reaction

Clinical sensitivity:

For the purposes of this evaluation is considered as clinical sensitivity the ability to determine true positives on the totality of positive samples screened. The analysis was performed on samples positive for HPV Low and High Risk and the test was performed following the instructions in the package insert. Positive samples were confirmed with another system on the market. Results obtained showed a clinical sensitivity of 97%.

Analytical specificity:

The specificity of the test is guaranteed by the use of specific primers for the determination of L1 region and E6/E7 region. The alignment analysis of the regions chosen for the hybridization of specific primers for Human Papilloma Virus with sequences available in database of L1 region and E6/E7 shows their conservation, the absence of significant mutations and the complete specificity for the target analyzed.

Diagnostic specificity:

For the purposes of this evaluation is considered as diagnostic specificity the ability of the method to determine true negative samples. The specificity of the diagnostic system was evaluated by analyzing human genomic samples tested and confirmed negative with another system on the market.

	Samples
N° of Tests	43
Positive n°	1
Negative n°	42

The results shows a Diagnostic specificity of 98%.

Reproducibility and Repeatability:

The reproducibility and repeatability of the system was assessed by analyzing 2 dilutions (High and Low) of plasmid DNA containing the genomic region of interest and quantified by spectrophotometric analysis (pHPV16) and 1 negative control (genomic DNA negative). 5 replicates are performed for each session for 3 different sessions performed by different operators.

		1	2	3	4	5
30000 cps	lot 0	+++	+++	+++	+++	+++
	lot 2	+++	+++	+++	+++	+++
	lot 3	+++	+++	+++	+++	+++
300 cps	lot 0	+++	+++	+++	+++	+++
	lot 2	+++	+++	+++	+++	+++
	lot 3	+++	+++	+++	+++	+++
Negative	lot 0	---	---	---	---	---
	lot 2	---	---	---	---	---
	lot 3	---	---	---	---	---

The results shows a reproducibility of 95%.

Cross-Reactivity:

The alignment analysis of the regions chosen for the hybridization of specific primers for Human Papilloma Virus

with sequences available in database of the region of L1 region and E6/E7 shows their conservation, the absence of significant mutations and the complete specificity for the target analyzed.

It was also carried out analysis on positive samples for other virus and the test was performed following the instructions for use.

Sample	Positive samples for	HPV CL07HR	HPV CL07HR	HPV CL07HR
1	HSV	Negative	Negative	Negative
2	CMV	Negative	Negative	Negative
3	EBV	Negative	Negative	Negative

INTERFERENCE

Verify that the extracted DNA from the sample base there are no mucoproteins and hemoglobin in order to exclude possible inhibition in the PCR reaction. Interference due to contaminants can be detected by spectrophotometric analysis and ratio of data obtained at 260 nm (absorption maximum of nucleic acids) and 280 nm (absorption maximum of proteins). A pure DNA should have a ratio of about 1.8.

QUALITY CONTROL

You should verify the correct extraction of DNA from test samples using the product CL54M - β -globin that allows the human β -globin gene amplification.

It's also suggested to include a negative and a positive sample tested already before or reference material of known, as an internal quality control of each session of extraction, amplification and detection.

REFERENCES

1. Fujinaga Y. et al (1991) *J Gen Virol* 72: 1039-1044
2. Karlsen F. et al (1996) *J Clin Microbiol* 34 (9): 2095-2100
3. Bouda M et al (2000) *Mod Pathol* 13 (6): 644-653
4. Soltar K. et al (2004) *J Clin Microbiol* 42 (7): 3176-3184

TROUBLESHOOTING

Amplification signal in the negative control reaction









Possible causes	Solutions
Error displacing the DNA extract.	<ul style="list-style-type: none"> - Use only micropipettes and Sterile tips with aerosol filter - Wear protective clothing such as laboratory coats and disposable gloves while assaying samples - Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use - Use the positive control as the last sample - Use the negative control as the first sample
Contamination of the amplification mixes	<ul style="list-style-type: none"> - Don't dispense the amplification mix with the some pipettes used in the extraction or detection area - Use only micropipettes and Sterile tips with aerosol filter - Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use - Close the amplification mix and return it at -20°C before open the others sample tubes
Contamination of the preparation area for amplification reaction	<ul style="list-style-type: none"> - Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use

Failure or incorrect amplification of positive control

Possible causes	Solutions
Possible error in the dispensation of the positive control	<ul style="list-style-type: none"> - Dispense carefully the positive control - Check the calibration of micropipettes used - Verify that the amount of positive control used corresponds to the method described in the Analytical Procedure (3μl)
Positive Control Degradation	<ul style="list-style-type: none"> - Test the mixture through a new batch of positive control
Wrong heat protocol	<ul style="list-style-type: none"> - Check the thermal cycler setting
The reaction mix is maintained at room temperature for too long	<ul style="list-style-type: none"> - Keep the amplification mix on ice during the use and store it at -20 ° C immediately after finishing the preparation of test samples
Error during the dispensation of the amplification product in the the detection phase	<ul style="list-style-type: none"> - Be careful when loading on the gel of amplified

Aspecific bands in the sample amplification reaction

Possible causes	Solutions
Wrong heat protocol	<ul style="list-style-type: none"> - Check the thermal cycler setting
Interfering Presence	<ul style="list-style-type: none"> - Repeat the extraction step carefully
Excess of extracted DNA in the reaction	<ul style="list-style-type: none"> - Evaluate the DNA concentration

	In vitro diagnostic device
	Read the instruction's manual
	Range of temperature
	Use within (dd/mm/yyyy: year-month)
	Lot (xxxx)
	Code
	Manufacturer
	Contains sufficient for <n> tests

EDMA code: 1504400300
CND: W0105040503



CLONIT S.r.l. via B. Quaranta 57
20139 Milano – Tel. 02-56.81.44.13

Printed: 12th July 2011

