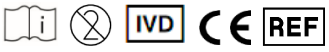
HPV Genotyping Real-time PCR Kit
Instructions for Use

For professional in vitro diagnostic use only.

BSJ01M1

48 Tests/Kit



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Effective Date: 2020-03-04

【Intended Use】

HPV Genotyping Real-time PCR Kit is an in vitro nucleic acid amplification test for the qualitative detection of human papillomavirus (HPV) type 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68; the kit can be used for detection but does not for differentiation between HPV type 26, 73 and 82.

The kit can be used for differentiation HPV types (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68). The kit can be used for the auxiliary diagnosis and epidemiological surveillance of HPV, cannot be used as the basis for the diagnosis or exclusion of cases alone.

For professional in vitro diagnostic use only.

【Principle】

HPV Genotyping Real-time PCR Kit is a qualitative, in vitro diagnostic test for the detection of HPV nucleic acid sequence of type 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68 from specimens. The conservative L1 sequence of late reading frame (about 1500bp) in human papillomavirus (HPV) is selected as the detection target. According to the nucleic acid sequence characteristics of each type, specific primers and fluorescent probes are designed to cover the target types of HPV genomes. In addition, the human genome β-Globin gene is introduced into the process as a non-competitive internal parameter to monitor the whole extraction and detection process. This kit can be used for assistant diagnosis of HPV genotype infection in clinic.

【Components】

Components		**Volume**	**Main Ingredients**
Amplification reagent	PCR Reaction Solution	960μL/tube, 2 tubes	Tap DNA polymerase, dNTP, PCR-buffer, etc.
	Detection Solution #1	240μL/tube, 1 tube	Primers and Probes for HPV 6, 11, 31, 59 and Internal Reference
	Detection Solution #2	240μL/tube, 1 tube	Primers and probes for HPV 16, 18, 35, 51 and 45
	Detection Solution #3	240μL/tube, 1 tube	Primers and Probes of HPV 33, 58, 52, 68 and 39
	Detection Solution #4	240μL/tube, 1 tube	Primers and Probes of HPV 53, 56, 26, 73, 82 and 66
Control	Positive control	240μL/tube, 1 tube	Recombinant plasmids of HPV 31, 16, 39 and 53
	Negative control	240μL/tube, 1 tube	Human Genome with Concentration of 2 ng/μL

a. The positive control and negative control need to be set to monitor the test body and the operating environment; the negative and positive control have been packaged in the kit.

b. The components of different lots cannot be mixed for use.

c. Equipment or materials required but not provided: Specimen collection kits, Nucleic acid extraction kits; PCR tubes and caps, etc.

【Applied instrument】

The kit can be applied to Bioer's Line-Gene 9600 Plus series fluorescent quantitative PCR detection system.

【Warnings and Precautions】

- For professional *in vitro diagnostic* use (IVD).Do not use after expiration date.
- Read the package insert carefully before performing the test. The appropriate operations from specimen collection, storage and transportation, and laboratory test should be strictly manipulated in line with relevant regulations of biosafety and molecular laboratory management.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled. Wash hands thoroughly after handling specimens and kit reagents.
- All the articles in each district are for special use which cannot allow to be exchanged for avoiding pollution. The workbench should be cleaned immediately after the completion of each experiment.
- Use disposable gloves without fluorescent substances, disposable special centrifuge tubes, etc.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents, while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- The false positive or negative testing result can be led by poor quality of specimen, incorrect operations in sample collection, transportation or laboratory processing, or limitation of the technology. Operator should understand well the principles of the procedures and its limitation in performance in advance and avoid any potential mistakes intentionally.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product.
- Separate laboratory areas are recommended to performing predefined procedures of the assay.
  Area I: Reagent preparation area-reagent required for preparing amplification.
  Area II: Sample processing area-processing of tested samples and controls.
  Area III: PCR detection region-PCR amplification detection.
- The separation of the reaction solution should avoid the generation of air bubbles as far as possible. Before the amplification, pay attention to check whether the caps of each reaction tube are tightened to avoid contaminating instrument.
- Samples should be completely put into the reaction solution when adding samples. No samples should adhere to the tube wall and the cap should be tightened as soon as possible after adding samples.
- The extracted nucleic acid sample should be used immediately after extraction.
- After amplification, please take out the reaction tube immediately, seal it in the s pecial plastic bag, put it in the designated place, and wait for unified treatment.
- Dispose of used / unused kit reagents and human specimens according to local, state, and federal regulations.

【Storage and period of validity】

1. The kit should be stored at -25℃ ~ -15℃ away from light.
2. Repeated freeze-thaw (more than 5 times) should be avoided.
3. The kit can be stored for up to 12 months if all components are kept in the manner above. Do not uses after the stated expiry date (indicated on the outside of the package).
4. The kit can be transported in foam box sealed with ice bags or dry ice at 2℃ - 8℃ or lower for up to 5 days.

【Specimen Collection, Storage, and Transportation】

1. Collection: Specimens can be collected by conventional methods.
2. Storage: It is recommended that specimens be processed as soon as possible after collection. If specimens are not processed immediately they should be stored at 2-8 ℃ for up to 3 days. If a delayed processing is expected, the specimens should be stored at -25℃ ~ -15℃ for up to 3 months. Specimens should not be frozen and thawed frequently.
3. Transportation: Specimen can be transported in foam box sealed with ice bags or dry ice at 2℃ - 8℃ or lower for up to 5 days.

【Specimen pretreatment (specimen disposal area)】

Follow the instructions of the nucleic acid extraction and purification kit.

It is recommended to use MagaBio plus Virus DNA/RNA Purification Kit (BSC57 & BSC71) to purify the nucleic acid. The Gene Pure Series Nucleic acid extractor is recommended to use to extraction nucleic acid automatically.

【Using of the kit - PCR reaction (PCR test area)】

Note: The negative control, positive control and unknown specimen need to be tested in the same experiment.

It's recommended to prepare the reagent ahead of specimen pretreatment to ensure that the reagents are not contaminated.

1) Reagent prepares

a. Thaw out the reagents at room temperature. Mix gently and centrifuge all reagents for a few seconds at 7000 rpm.

b. Calculate the amount of PCR Reaction Solution and detection solution according to the quantity of specimens and controls as below (N means the number of specimens and controls. Extra blank positive control(s) and extra blank negative control(s) are highly recommended to prevent the loss of reaction mix.):

Reagents	Vial #1	Vial #2	Vial #3	Vial #4
PCR Reaction Solution	(N+2) ×10μL	(N+2) ×10μL	(N+2) ×10μL	(N+2) ×10μL
Detection solution	Detection Solution #1 (N+2) ×5μL	Detection Solution #2 (N+2) ×5μL	Detection Solution #3 (N+2) ×5μL	Detection Solution #4 (N+2) ×5μL

Note: 1. Due to the high viscosity of PCR reaction liquid, attention should be paid for the residual of the pipetting. It's highly recommended to enlarge the amount of formulation appropriately, when the specimen number is high.

2. For each experiment set, an extra blank positive control and an extra blank negative control should be introduced.

c. Prepare the solutions in accordance with the calculated amount.

d. Mix the PCR Reaction Solution and detection solution gently, and centrifuge the mixed reagents for a few seconds at 7000 rpm. **(**Detection Tube #1 and PCR Reaction Solution should be mixed and added into vial#1; Detection Tube #2 and PCR Reaction Solution should be mixed and added into vial#2; Detection Tube #3 and PCR Reaction Solution should be mixed and added into vial#3; Detection Tube #4 and PCR Reaction Solution should be mixed and added into vial#4.)

e. Distribute 15μL mixed reagents (in vial#1, vial#2, vial#3 and vial#4) into PCR tubes, and then transfer the reaction plate to sample processing area.

2) Adding sample

a. Deem each four PCR test contain reagent of Vial# 1, Vial# 2, Vial# 3 and Vial# 4 respectively as a test team for a single specimen or a control.

b. Add 5μL negative control and 5μL positive control in each PCR reaction wells of a test tube team respectively.

c. Add 5μL extracted products in each PCR tubes of a test tube team respectively.

d. Cap the PCR tube(s) immediately to prevent cross contamination. DO NOT LABEL ON THE SCANNED AREA OF THE REACTION TUBES!

e. Instantaneous centrifugation until there is no liquid on the wall of tubes and no bubbles in the solution. Positive control and negative control need to be introduced into each PCR test reaction.

3) RT-PCR reaction

Place the reaction tubes on a RT-PCR instrument.

It is recommended to choose FAM, HEX, ROX, Cy5 and Cy5.5 channels to collect fluorescent signals. Gain value of FAM channel should be set at 10; gain value of HEX channel should be set at 8; gain value of ROX channel should be set at 10; gain value of Cy5 channel should be set at 12; gain value of Cy5.5 channel should be set at 12. Baselines and threshold value line should be set at automatic.

Set fluorescent signals detecting at 60℃, liquid volume is 20μL.

Set reaction procedure as below:

Step	Temperature	Duration	Number of cycles
1	95℃	3 min	1
2	95℃	10 sec	40
3	60℃*	15 sec	

Note: Fluorescence signals need to be collected in the step with *mark.

【Quality control standards】

Expected performances of controls are as below:

		FAM	**HEX**	**ROX**	**Cy5**	**Cy5.5**
Negative control	Vial #1	None detected	None detected	None detected	Standard ‘S’ amplification curve	None detected
	Vial	None	None	None	None	None

	#2	detected	detected	detected	detected	detected
	Vial #3	None detected	None detected	None detected	None detected	None detected
	Vial #4	None detected	None detected	None detected	None detected	None detected
Positive control	Vial #1	None detected	None detected	Ct Value≤30	Ct Value≤30	None detected
	Vial #2	None detected	Ct Value≤30	None detected	None detected	None detected
	Vial #3	None detected	None detected	None detected	None detected	Ct Value≤30
	Vial #4	Ct Value≤30	None detected	None detected	None detected	None detected
Specimen(s)	Vial #1				Ct Value≤ 38.2	

Note: All the requirements above should be met in each test; otherwise, the test is invalided.

【Result Analysis and Judgments】

According to the results of clinical trials, the qualitative reference values of each subtype detected by this kit are obtained by ROC curve method, as shown in the following table:

HPV Result Analysis Interpretation Table and Reference Value

Vial	HPV Subtype	Detection Channel	Reference Value
Vial#1	6	FAM	39.5
	11	HEX	38.8
	31	ROX	39.1
	Internal Reference Genes	Cy5	38.2
	59	Cy5.5	38.6
Vial#2	18	FAM	35.4
	16	HEX	34.5
	35	ROX	36.9
	45	Cy5	39.3
	51	Cy5.5	38.1
Vial#3	33	FAM	37.1
	58	HEX	35.3
	52	ROX	35.2
	68	Cy5	35.4
	39	Cy5.5	35.5
Vial#4	53	FAM	38.4
	56	HEX	34.3
	26, 73, and 82	ROX	38.3
	66	Cy5	34.6

NOTE:

1. All the requirements list in the Quality Control Standard should be met, then the results of can

be interpreted and analyzed.

2. It can be deemed as positive for a specific HPV subtype that a standard 'S' amplification curve is detected with the Ct value no more than the reference value list above for a specific channel in a specific vial.
3. It's recommended to retest that a standard 'S' amplification curve is detected with the Ct value higher than the reference value list above for a specific channel in a specific vial. It can be deemed as positive for a specific HPV subtype if the retest result is consistent with the former result. Otherwise, it can be deemed as negative for a specific HPV subtype.
4. It should be deemed as negative for a specific HPV subtype that no standard 'S' amplification curve is detected.
5. Special instructions: it should be deemed as medium-risk type positive that a standard 'S' amplification curve is detected with the Ct value no more than 38.3 for ROX channel in Vial#4. It means that at least one type from 26, 73 and 83 is positive.

【Limitations】

1. HPV Genotyping Real-time PCR Kit is an in vitro nucleic acid amplification test for the qualitative detection of human papillomavirus (HPV) type 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68; the kit can be used for detection but does not for differentiation HPV type 26, 73 and 82. The kit does not detect HPV subtypes which are not mentioned above. The test kit does not detect HPV type 42, 43, 54, 61, 72, and 81.
2. The results of the test are just for clinical reference. The test should not be used as sole criteria for diagnosis. Results should be considered in conjunction with the clinical information and other data available to the physician.
3. Due to the limitation of detection threshold and detection range, negative results do not preclude infection with HPV and should not be the sole basis of a patient management. Follow up testing/ analysis should be performed.
4. False negative or false positive result may occur by incorrect operation in sample collection, transportation, processing; aerosol pollution or operating errors.

【Performance Indicators】

★ Analytical sensitivity: 20 HPV positive controls standardized by national standard which contain all the detecting subtype of the kit were tested. The positive coincidence rate was 100%.

★ Analytical specificity: No cross reactivity has been observed for the HPV subtypes and specimens list below.

HPV subtypes 42, 43, 54, 61, 72, and 81 (at the concentration of 10^6~10^7 copies/mL).

Specimens: herpes simplex virus type II, Treponema pallidum, Ureaplasma urealyticum, gonococcus, Candida albicans, Trichomonas vaginalis, Chlamydia trachomatis.

Interfering Substances: Blood, cervical mucus, human lubricant, vaginal wash, miconazole nitrate, phenylmercury acetate.

★ Precision: the variation coefficient (CV) of within-lot, between-lots, between-operators and between-days were less than 5%.

【References】

[1] State Food and Drug Administration Decree No. 6 "Guiding Principles for the Preparation of

Instructions for in vitro Diagnostic Reagents".

[2] Schlecht NF,Trevisan A, Duarte-Franco E, et al. Viral load as a predictor of the risk of cervical intraepithelial neoplasia [J]. Int J Cancer, 2003,103(4):519-524.

[3] Moberg M, Gustavsson I, Glyllensten U. Real-time PCR-based system for simultaneous quantification of human papillomavirus types associated with high risk of cervical cancer [J]. J Clin Microbiol, 2003, 41(7):3221-3228.

[4] Castle PE, Solomon D, Schiffman M, et al. Wheeler for the ALTS group human papillomavirus type 16 infection and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities [J]. J Natl Cancer Inst, 2005,97(14):1066-1071.

【Symbol Description】

CE	**CE MARK**	IVD	**IN VITRO DIAGNOSTIC MEDICAL DEVICE**
	**CAUTION**		**MANUFACTURER**
	**CONSULT INSTRUCTIONS FOR USE**		**DATEOF MANUFACTURE**
	**TEMPERATURELIMITATION**		**USE BY DATE**
EC REP	**AUTHORISED REPRESENTATIVE IN THE EUROPEAN COMMUNITY**		**DO NOT REUSE**

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EC REP

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