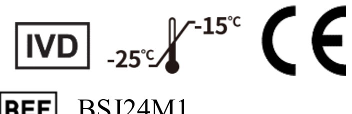
HPV (6/11/16/18) Genotyping Real-Time PCR Kit

Instructions for Use



BSJ24M1

Effective Date: Jan 10, 2022

For professional use only.

For in vitro diagnostic use only.

INTENDED USE

HPV (6/11/16/18) Genotyping Real-Time PCR Kit is an in vitro diagnostic test for the qualitative detection of DNA from Human Papillomavirus (HPV)type 6, type 11, type 16 and type 18 in specimens of cervical swab.

HPV (6/11/16/18) Genotyping Real-Time PCR Kit specific identifies HPV type 6, type 11, type 16 and type 18, it can’t be used for detection of the other HPV types. The kit is used for the auxiliary identification diagnosis and epidemiological surveillance of HPV-6/11/16/18 infection, cannot be used as the basis for the diagnosis or exclusion of cases alone.

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PRINCIPLE

HPV (6/11/16/18) Genotyping Real-Time PCR Kit based on in vitro Real time PCR combining fluorescent probing. Primers and a sequence-specific fluorescence probes were designed tailored to L1 gene of HPV genome. The probes of HPV 18 are oligonucleotide attached fluorophores at the 5' end with FAM; the probes of HPV 16 are oligonucleotide attached fluorophores at the 5' end with HEX; The probes of HPV 6 are oligonucleotide attached fluorophores at the 5' end with ROX; The probes of HPV 11 are oligonucleotide attached fluorophores at the 5' end with Cy5.5. In a meantime, specific primers and probes on basis of human β-Globin gene were developed as internal reference with fluorophores CY5 attached at 5’ end as reporter. All of probes 3’ end with quencher. During the PCR procedures, the DNA polymerase cleaves the probe at the 5’ end and separates the reporter dye from the quencher dye when the probes hybridize to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. Monitoring the fluorescence intensities during Real Time allows the qualitative detection of HPV (6/11/16/18) in specimens.

COMPONENTS

Components		BSJ24M1	Main Ingredients
Kit size		48 tests/kit	
Amplification reagent	PCR Reaction buffer	480μL×1	dNTP, Mg^{2+}, Tris
	Detection Solution	240μL×1	DNA polymerase, Specific Primers and Probes
Control	Positive control	300μL×1	Solution containing HPV 6/11/16/18 L1 gene plasmid
	Negative control	300μL×1	Solution containing internal reference gene plasmid

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. pipette and pipette tips, vortex, etc.*

APPLIED INSTRUMENT

The kit can be applied to Hangzhou Bioer Technology Co., Ltd. fluorescent quantitative PCR detection system, Quantgene 9600 (FQD-96C). The instrument should contain at least five channels of FAM, HEX, ROX, CY5 and Cy5.5.

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 pipette by mouth. 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. Avoid DNA contamination of reagent.
- 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°C ~ -15°C away from light and avoid repeated freeze-thaw. The kit can be stored for 3 days at 2-8 °C after opening.
2. The kit can be stored for up to 12 months if all components are kept in the manner above. Do not use after the stated expiration date.
3. The kit can be transported in foam box sealed with ice bags or dry ice at not higher than 8°C.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORTATION

1. Specimen: Cervical swab specimens

2. Collection: Cervical specimens can be collected by conventional methods with a disposable sterile cervical swab. Insert the cervical swab tip into a sample preservative fluid and shake to release the cervical specimen.
3. 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 °C for up to 24hours. If a delayed processing is expected, the specimens should be stored at -70°C or lower. Specimens should not be frozen and thawed frequently.
4. Transportation: Specimen should be transported with 0°C curling bottle or foam box sealed with ice.

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 III** (BSC86) to purify the nucleic acid. The Gene Pure Series Nucleic acid extractor is recommended to use to extraction nucleic acid automatically.

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.

USING OF THE KIT - PCR REACTION (PCR TEST AREA)

1) Reagent prepares

Thaw out the reagents at room temperature. Gently mix and centrifuge all reagents for a few seconds.
Make PCR reagents according to the quantity of specimens and controls as below (N means the number of **specimen(s) and controls**. An extra blank control is highly recommended to prevent the loss of reaction mix.):

Reagents	PCR reaction buffer	Detection solution
Dosage/ test	10μL	5μL
Dosage	(N+1) ×10μL	(N+1) ×5μL

Distribute 15 μL mixed PCR reagents into each PCR tubes, and then transfer the reaction plate to sample processing area.

2) Adding sample

Add 5μL negative control, 5μL extracted product, 5μL positive control into different PCR tubes. Cap the PCR tubes immediately to prevent cross contamination.

Note: Do not label on the scanned area of the reaction tubes!

3) PCR reaction

Place the reaction tubes on a PCR instrument.

It is recommended to choose FAM, HEX, ROX, CY5 and Cy5.5 channels to collect fluorescent signals.

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

Set reaction procedure as below:

Step	Temperature	Duration	Number of cycles
1	95°C	1 min	1
3	95°C	15 sec	40
	60°C	20 sec	

QUALITY CONTROL STANDARDS

Expected performances of controls are as below:

	FAM	HEX	ROX	CY5	Cy5.5	Interpretation of Test Results
Positive Control	Ct Value ≤30	Ct Value ≤30	Ct Value ≤30	Ct Value ≤30	Ct Value ≤30	All requirements are met in the same experiment, indicating that the experiment is valid, otherwise it is invalid.
Negative Control	No Ct Value			Ct Value ≤30	No Ct Value	

RESULT ANALYSIS AND JUDGMENTS

Expected performances of specimens are as below:

Channels	Ct value	CY5 Ct Value	Result Judgment
FAM	Ct Value ≤35	Ct Value ≤35	HPV-18 nucleic acid Positive
	Ct Value >35		Retest the sample. If the results are consistent, it means that HPV-18 nucleic acid positive; Otherwise, it is negative.
HEX	Ct Value ≤35		HPV-16 nucleic acid Positive
	Ct Value >35		Retest the sample. If the results are consistent, it means that HPV-16 nucleic acid positive; Otherwise, it is

			negative.
ROX	Ct Value ≤35		HPV-6 nucleic acid Positive
	Ct Value >35		Retest the sample. If the results are consistent, it means that HPV-6 nucleic acid positive; Otherwise, it is negative.
Cy5.5	Ct Value ≤35		HPV-11 nucleic acid Positive
	Ct Value >35		Retest the sample. If the results are consistent, it means that HPV-11 nucleic acid positive; Otherwise, it is negative.

Note:

1. Result consistent means the repeat test yields obvious S-shaped amplification curve.

2. When the Ct value of CY5 channel is more than 35, the result cannot be interpreted, indicating that there is a problem in sample quality or extraction. It is recommended to re-extract or re-collect specimen.

LIMITATIONS

1. Test only the indicated specimen type. This kit is an in vitro nucleic acid amplification test for the qualitative detection of human papillomavirus (HPV) type 6, 11, 16 and 18. The kit does not detect HPV subtypes which are not mentioned above.
2. Use of the product must be limited to personnel trained in the techniques of PCR and the use of applicable instrument.
3. 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.
4. 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.
5. False negative or false positive result may occur by incorrect operation in sample collection, transportation, processing, aerosol pollution or operating errors.

PERFORMANCE INDICATORS

Performance validation was conducted with the Quantgene 9600 series fluorescent quantitative PCR detection system from Bioer. The kit can be applied to Bioer's Quantgene 9600 series fluorescent quantitative PCR detection system and other manufacturers' similar fluorescent quantitative PCR detection systems.
Since positive specimen of HPV (6/11/16/18) was unavailable, positive control was prepared for the validation. The positive control was trace back to HPV L1 gene National reference (National Institutes for Food and Drug Control). which contains HPV-6, HPV-11, HPV-16 and HPV-18 L1 gene.

★ Limit of Detection (LoD): The positive reference standard was diluted into 2000 copies/mL, 1000 copies/mL, 500 copies/mL and 250 copies/mL, then were tested by 3 lots of kits. Each control was tested with 20 replicates. The testing data demonstrated that the kit can detect the HPV (6/11/16/18) with detection rate equal or higher than 95% at the concentration equal or higher than 1000 copies/mL.

★ Analytical sensitivity: 3 positive reference standards and 4 negative reference standards were tested by 3 lots of kits. The positive coincidence rate was 100%, and the negative coincidence rate was 100 %.

★ Analytical specificity: No cross reactivity has been observed for the HPV subtypes and specimens list below HPV subtypes 26, 31, 33, 35, 39, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 72, 73, 81and 82 (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: Positive controls and low positive controls were tested by 3 lots of kits with 10 replicates by 2 operators for 20 days. The results showed that 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"
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[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

Symbol	Description	Symbol	Description
	Manufacturer	REF	Catalogue number
CE	CE mark	EC REP	Authorized representative in the European community
LOT	Batch code		Consult instructions for use
IVD	In vitro diagnostic medical device		Temperature limitation
	Caution		Use by date
CONTROL +	Positive Control	CONTROL -	Negative Control

HANGZHOU BIOER TECHNOLOGY CO., LTD.
1192 BinAn Rd., Binjiang District, 310053 Hangzhou, China
Website: www.bioer.com.cn
TEL: +86-571-87774575 FAX: +86-571-87774565

MedNet EC-REP GmbH
Borkstrasse 10, 48163 Muenster, Germany

TECHNICAL SUPPORT

Please dial phone number +86-571-87774567-5211 or 87774575, by fax to +86-571-87774553, or by email to reagent@bioer.com.cn.