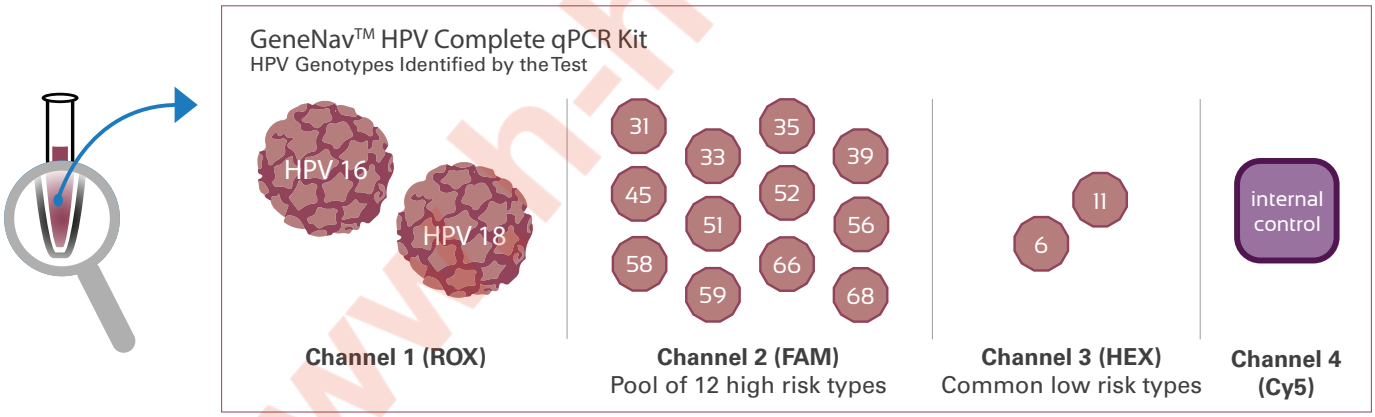
GeneNav™
HPV Complete qPCR Kit

IVD CE

REF E116

Introduction

Over 130 HPV types have been documented in literature, approximately 40 of which infect the anogenital area and are sexually transmitted. Persistent anogenital high risk HPV infection is associated with the majority of cervical cancers. Cervical cancer is highly preventable when cytological and HPV screening programs are employed to facilitate early detection and treatment of pre-cancerous lesions. Of the sexually transmitted HPV genotypes, 14 (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are considered high risk as they lead to cervical cancer or anogenital warts. In addition, HPV types 6 and 11 are considered low risk for cervical cancer, however they are the cause of 90% of all cases of genital warts as well as respiratory papillomatosis. The GeneNav™ HPV Complete qPCR Kit is a single tube system which utilizes quantitative PCR (qPCR) technology to detect the 16 HPV types described. This kit is able to simultaneously detect the presence of HPV 16 or HPV 18, one of the other high risk HPV subtypes, and HPV 6 or 11 (refer to figure below).



Kit Components

PN Part Number	Kit Components	Quantity
E-1S	Diagnostic 2X qPCR MasterMix – No Dye	500 µl
E116-A	GeneNav™ HPV Complete Primers	50 µl
E116-B	GeneNav™ HPV Complete Probes	50 µl
E10	Sample Prep Solution	10 ml
E0	Nuclease-free Water	1 ml

- For 48 tests
- Store all reagents between -25°C to -15°C in a non-frost-free freezer

Version: 2016/10/25

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Principles of the Assay

The GeneNav™ HPV Complete qPCR Kit uses probe-based qPCR technology to detect HPV with high specificity. Each sequence-specific probe contains a 5' fluorophore and a 3' quencher. On its own, the probe's 3' quencher effectively absorbs the emission from the nearby 5' fluorophore so that no net fluorescence is detected. During PCR amplification, the probe binds to its specific target template. While replicating the DNA sample, the PCR Taq Polymerase's 5' to 3' exonuclease activity also hydrolyzes the probe, releasing a free-floating 5' fluorophore that is detectable by the qPCR machine and can be translated into an amplification plot. This HPV Complete qPCR kit identifies 14 types of high risk HPV, 2 types of low risk HPV, and a human ACTIN control using 4 fluorescent channels in one tube: ROX, FAM, HEX and Cy5. As a result, this kit can not only detect the 14 high risk HPV types and 2 low risk HPV types, it is also capable of distinguishing HPV 16 and 18 from all the other types. The internal control, human ACTIN, indicates the quality of extracted DNA.

Sample Requirements

Specimens are limited to cervical cells collected through a cervical swab. For the GeneNav™ HPV Complete qPCR Kit, cervical specimens can be stored in the Specimen Transportation Medium for up to 3 days (between 2 to 8°C) or up to 6 months (at -20°C) prior to testing. Avoid repeated freeze/thawing of the samples.

DNA Isolation

1. Transfer 500 µl of freshly re-suspended Specimen Transport Media/Sample mixture into an Eppendorf tube. Centrifuge the mixture at 12000 rpm for 5 minutes. Discard the supernatant.
2. Thoroughly re-suspend the Sample Prep Solution (E10) and immediately transfer 200 µl of the homogenized Sample Prep Solution (E10) to the collected cell pellet.
3. Vortex the mixture thoroughly. Heat the mixture at 100°C for 10 minutes then cool the mixture on ice for 5 minutes.
4. Centrifuge the mixture at 12000 rpm for 5 minutes. The resulting supernatant is used directly as the template in the ensuing analyses and will be referred to as the "extracted DNA".

note If the supernatant is transferred into another tube, it may be stored up to one year at -20°C.

Quantitative PCR (qPCR) Reaction Setup

1. Thaw the following reagents on ice: Diagnostic 2X qPCR MasterMix - No Dye (E-1S), GeneNav™ HPV Complete Primers (E116-A), and GeneNav™ HPV Complete Probes (E116-B). Gently and evenly mix each individual reagent, then briefly centrifuge the reagents to collect the contents.

2. Set up a premix solution based on the number of sample preps to be tested. The volume of the premix required for all sample prep(s) to be tested = (number of sample preps + 1) X the total volume of premix reagents (listed in Table 1).

Table 1: Premix Reagents	Volume
Diagnostic 2X qPCR MasterMix - No Dye (E-1S)	10 µl
GeneNav™ HPV Primers (E116-A)	1 µl
GeneNav™ HPV Probes (E116-B)	1 µl
Nuclease-free Water (E0)	6 µl
Total Volume	18 µl

3. Evenly aliquot the premix(es) into qPCR tube(s) (one qPCR tube per sample to be tested). Add 2 µl of each extracted DNA solution (refer to Step 4 of DNA Isolation protocol) to a single qPCR tube. Do not add more than one sample of extracted DNA into a single qPCR tube. Use the Nuclease-free Water (E0) instead of extracted DNA for the No Target Control (NTC).

   Control: The NTC must be run on each assay plate, and the cycle threshold (CT) for the NTC must be more than 37 for the sample(s) on the plate to be valid. If this criteria is not met, the sample(s) and controls on that plate are invalid and must be repeated.

qPCR Cycling Condition

Transfer the reaction setup into a qPCR machine and set up the following thermal cycling program. It is recommended to use BioRad's CFX96, Applied Biosystem's 7500, or QuantStudio's 7 Flex system.

DNA Pre-denaturing	95°C, 10 min	1 Cycle
Denaturing	95°C, 15 sec	40 Cycles
Anneal / Extension	61°C, 60 sec (Collect ROX, FAM, HEX, & Cy5 signals)	

note Signals are collected at the end of each extension step through ROX, FAM, HEX, and Cy5 channels. The ROX passive reference MUST be turned OFF.

Detection Channels

Four channels (FAM, HEX, ROX, & Cy5) are used in this one-tube qPCR assay. It is recommended to perform the color (channel) calibration as requested by the instrument's manufacturer. Please refer to the instrument's user manual to perform this calibration.

Choose the FAM, HEX, ROX, and Cy5 channels for each sample to be tested with GenomeMe™'s GeneNav™ HPV Complete qPCR Kit. Select "None" for ROX passive reference on any Applied Biosystem's qPCR machine.

Result Analysis

1. If the internal control (Cy5) shows a CT value of greater than 37, the test has failed due to insufficient amounts of DNA or poor quality DNA. Re-extract DNA and repeat the assay.
2. If the internal control (Cy5) shows a CT value of less than 37, and all other HPV types (ROX, FAM, and HEX) show a CT value of greater than 37, the sample is negative for all 16 types of HPV that this kit detects.
3. If both the internal control (Cy5) and any one of FAM, HEX, or ROX channels show a CT value of less than 37, the sample is positive. The ROX channel indicates the sample is HPV 16 or 18 positive, the FAM channel indicates it is positive for any of the following HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, and the HEX channel indicates it is positive for HPV 6 or 11.

note Sample results are only valid when negative controls yield no amplification. If the NTC is invalid in an assay, all sample results in the same assay are therefore also invalid and the assay must be repeated.

Performance Characteristics

The following performance characteristics of the GeneNav™ HPV Complete qPCR Kit have been established following the procedure described in this datasheet.

Analytical Sensitivity: 130 copies/reaction for all 16 types.

Analytical Specificity: Cross reaction among these 16 HPV types was not observed. A panel of bacteria, fungi, and viruses commonly found in the female anogenital tract were tested with the GeneNav™ HPV Complete qPCR Kit to assess potential cross-reactivity. The pathogens tested include: *Candida albicans, Proteus vulgaris, Corynebacterium pseudodiptheriticum, Staphylococcus aureus, Enterococcus faecalis, Staphylococcus epidermidis, Escherichia coli, Streptococcus mitis, Lactobacillus acidophilus, Streptococcus pyogenes, Herpes simplex virus, type 1 and 2 (HSV-1 and 2), Chlamydia trachomatis, Neisseria gonorrhoeae, Human Immunodeficiency Virus type 1 (HIV-1 pol and env regions), and Mycoplasma hominis.* Negative results were obtained from all above-mentioned organisms.

Reproducibility: Reproducibility of the GeneNav™ HPV Complete qPCR Kit was assessed at two external sites using a panel of HPV positive and negative cultured cells and HPV positive and negative cervical specimens. DNA was extracted from 500 µl of cervical samples or cultured cells suspended in Specimen Transportation Medium. The DNA was extracted using the sample prep solution included in this Kit. DNA was extracted from sixteen samples and tested with GeneNav™ HPV Complete qPCR Kit at two sites on five non-consecutive days within a two-week time period. Two lots of GeneNav™ HPV Complete qPCR Kits were used across the two sites for the study. The total number of measurements for each sample was 20 (2 sites x 5 days x 2 lots x 1 run per day). The results showed that the coefficient of variation (CV) for the kit performance was less than 5% among different product lots and different operators.

Limitations

1. The GeneNav™ HPV Complete qPCR Kit detects DNA of 16 HPV types (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).
2. A negative result does not exclude the possibility of HPV infection because very low levels of infection or sampling error may cause a false-negative result.
3. The test has been validated for use only with cervical swab specimens collected in specimen transportation medium.
4. The performance of the GeneNav™ HPV Complete qPCR Kit was established exclusively using DNA extracted with the enclosed sample prep solutions.
5. The performance of the GeneNav™ HPV Complete qPCR Kit has not been adequately established for HPV vaccinated individuals.
6. Prevalence of HPV infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.
7. Infection with HPV is not an indicator of cytologic high-grade squamous intraepithelial lesion (HSIL) or underlying high-grade cervical intraepithelial neoplasia (CIN). Nor does it imply that CIN2-3 or cancer will develop. Most women infected with one or more HPV types do not develop CIN2-3 or cancer.
8. A negative result for high-risk HPV types does not exclude the possibility of future cytologic HSIL or underlying CIN2-3 or cancer.

Disclaimer

INTENDED USE:

1. To diagnose and identify the 14 high risk and 2 low risk types of HPV infection in patients, while distinguishing HPV 16 and HPV 18 from the other 16 HPV types.
2. In women 30 years and older, the GeneNav™ HPV Complete qPCR Kit can be used with a cervical cytology to adjunctively assess the presence or absence of HPV infection. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

WARNINGS:

1. The GeneNav™ HPV Complete qPCR Kit is not intended for use as a screening device for women under age 30 with normal cervical cytology.
2. The GeneNav™ HPV Complete qPCR Kit is not intended to substitute for regular cervical cytology screening.
3. The use of this test has not been evaluated for the management of women with prior cytological or histological abnormalities, have had a hysterectomy, who are pregnant, postmenopausal, or who have other risk factors (e.g. HIV+, immunocompromised, history of STI).
4. The GeneNav™ HPV Complete qPCR Kit is designed to enhance existing methods for the detection of cervical disease and should be used in conjunction with clinical information derived from other diagnostic and screening tests, physical examinations, and full medical history in accordance with appropriate patient management procedures.
5. The GeneNav™ HPV Complete qPCR Kit results should not be used as the sole basis for clinical assessment and treatment of patients.

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