IntelliPlex™ HPV bDNA Genotyping Kit User Manual

REF **82017** **96 Reactions**

CE IVD **For In-Vitro Diagnostic Use**

PlexBio Co., Ltd.
6F-1, No. 351, Yangguang St., Neihu Dist.
Taipei City, 11491, Taiwan
886-2-2627-5878
http://www.plexbio.com

Co-branding with
DiaCarta, Inc.
2600 Hilltop Drive, Richmond, CA 94806
1-800-246-8878
information@diacarta.com

European Authorized Representative:
EC REP Medical Device Saftey Services GmbH (MDSS)
Schiffgraben 41, 30175, Hannover, Germany

IMPORTANT:
Read the instructions carefully prior to use

1. INTENDED USE

The IntelliPlex HPV bDNA Genotyping Kit is an in vitro assay intended for qualitative identification of HPV genotypes 16 and 18 while simultaneously detecting other 12 high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) from cervical specimens. It is designed to use πCode™ technology and PlexBio's instrument platform in conjunction with branched DNA (bDNA) detection system for the identification of HPV E6/E7 mRNA without target amplification. The cervical specimens should be collected from PreservCyt Solution. Together with the physician's assessment of cytology history, other risk factors, and professional guidelines, the result is intended to determine the need for referral to colposcopy.

2. INTRODUCTION

Human papillomavirus (HPV) is a common sexually transmitted DNA virus that infects the genital area of men and women. Some HPV types are classified as high-risk based on their association with cervical cancer and its precursor lesion, high-grade cervical intraepithelial neoplasia (CIN 2/3) in women. Patients with persistent infection with one of the genotypes considered high-risk for cervical disease have an increased risk for developing severe dysplasia or cervical carcinoma[1-2]. The presence of HPV nucleic acid does not necessary lead to cervical dysplasia or cervical cancer. The expression of the oncogenic elements of HPV is a better indication for the likelihood of persistent viral infection and cellular transformation. Two of the primary oncoproteins expressed in high risk HPV are E6 and E7. Expression of E6/E7 significantly lower the cellular levels of p53 and pRb, both are tumor suppressors in mammalian cells[3-5]. The IntelliPlex HPV bDNA Genotyping Kit is designed to detect the presence of E6/E7 mRNA using branched DNA (bDNA) technology. bDNA incorporates a series of probes that bind to multiple regions of the target mRNA as well as to the πCode MicroDisc. Subsequent hybridization of pre-amplifiers, amplifiers, and signaling molecules enables fluorescent detection of the target mRNA without the need for PCR amplification. Simultaneous detection of HPV 16, 18 and other 12 high risk genotypes in cervical specimens is feasible based on the optimal probe design and πCode technology. The IntelliPlex HPV bDNA Genotyping Kit identifies 14 HPV high-risk types, as listed in Table 1.

Table 1: Genotypes of Detected HPV

Risk Level	Subtypes
High-risk	**16, 18, and other 12 high-risk types** (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)

3. TECHNOLOGICAL PRINCIPLES

The IntelliPlex HPV bDNA Genotyping Kit utilizes two technologies- πCode and Branched DNA (bDNA) technologies - for detection of HPV 16, 18 and other 12 high-risk genotypes in one well reaction.

πCode MicroDisc

πCode MicroDisc is designed to generate up to 16,000 distinct circular image patterns for multiplexing applications. Each πCode has a distinct circular image pattern, which corresponds to a specific capture agent conjugated to the surface of the disc. All capture agent tagged πCode are pooled, enabling capturing and detection of specific analytes in one reaction.

Branched DNA Technology

The bDNA technology is achieved by signal amplification on the bDNA probe after direct binding of a large hybridization complex to the target sequence.[6-7] This series of hybridization steps results in a "sandwich" complex of probes and target sequence. Thus, the presence of E6/E7 mRNA in cervical samples collected from Hologic® ThinPrep® Pap Test (PreservCyt® Solution) is detected by using the bDNA technology.

Detection Principle

The test is achieved by a series of hybridization steps as shown as Fig. 1:

I. Specimen Lysis and First Hybridization: with HPV bDNA πCode MicroDiscs and HPV WR (Label extender, Capture extender and Blocking probes)
II. Second Hybridization: HPV A1 buffer (Pre-Amplifier)
III. Third Hybridization: HPV A2 buffer (Amplifier)
IV. Fourth Hybridization: HPV A3 buffer (Label Probe)
V. Incubation for SA-PE and fluorescence conjugation
VI. Image pattern decoding and fluorescent signal detection by the PlexBio™ 100 Fluorescent Analyzer

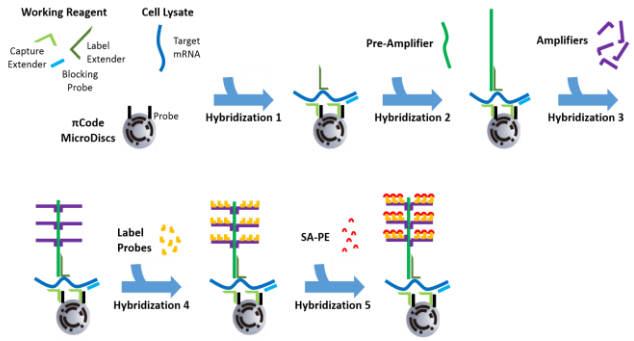


Fig 1. Hybridization steps of bDNA technology

4. WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- This assay kit should be used by qualified laboratory personnel only.
- Do not use a kit or reagent past its expiration date.
- Component reagents have been diluted optimally. Further dilution of the component reagents is not recommended.
- Specimens should be handled as infectious material. Please follow universal precaution for safe use.
- Store assay kits and reagents according to the product label and instructions.
- Do not mix reagents from different lots.
- Dispose of unused reagents, specimens and waste according to applicable central/federal, state, and local regulations.
- Wear powderless gloves and do not touch and make any markings on the bottom of the plate at any time, as fingerprints and markings would interfere with decoding and signal acquisition.
- General laboratory precautions should be taken:
  - Do not pipette by mouth.
  - Wear protective clothing (e.g., disposable powderless gloves and laboratory coats) and eye protection.
  - Do not eat, drink or smoke in the laboratory.
  - Wash hands thoroughly after handling samples and reagents.
- The workspace including racks and pipettes should be thoroughly cleaned and wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.
- Material Safety Data Sheets (MSDS) are available upon request from PlexBio Customer Service.

5. PRODUCT USE LIMITATIONS

- The test has only been validated for use with specimens collected by Hologic® ThinPrep® Pap Test (PreservCyt® Solution).
- The first hybridization procedure should be performed at exactly 53°C to prevent false positive or false negative results.
- Reliable results are dependent on adequate cervical specimen collection.

6. QUALITY CONTROL

The IntelliPlex HPV bDNA Genotyping Kit contains a series of πCode MicroDiscs as internal control to monitor the sample processing, SA-PE incubation procedure and background noise. The external controls (positive control and negative control) monitor the whole testing procedure to prevent false positive or false negative results. If the external positive or negative control did not meet the specified value, the test is considered invalid.

7. KIT COMPONENTS

The IntelliPlex HPV bDNA Genotyping Kit contains sufficient reagents for up to 96 tests. The kit components supplied are listed as follows.

1. **HPV bDNA WR**
   Ref. No.: 20069
   Quantity & Volume: 1 bottle, 9.12 mL/bottle
   Description: Buffer solution containing label extender, capture extender and blocking probe
   Contents: <50 mM Tris-HCl, <10 mM EDTA, <5% SDS, <500 mM LiCl, <0.1%Oligo mixture

2. **HPV bDNA A1 Buffer**
   Ref. No.: 20070
   Quantity & Volume: 1 bottle, 4.8 mL/bottle
   Description: Buffer solution containing Pre-Amplifier
   Contents: 0.1% sodium N-lauroyl sarcosinate, 30% Tetramethyl-ammonium Chloride,
   <0.1% sodium azide

3. **HPV bDNA A2 Buffer**
   Ref. No.: 20071
   Quantity & Volume: 1 bottle, 4.8 mL/bottle
   Description: Buffer solution containing Amplifier
   Contents: 0.1% sodium N-lauroyl sarcosinate, 30% Tetramethyl-ammonium Chloride,
   <0.1% sodium azide

4. **HPV bDNA A3 Buffer**
   Ref. No.: 20072
   Quantity & Volume: 1 bottle, 4.8 mL/bottle
   Description: Buffer solution containing label probes
   Contents: 0.1% sodium N-lauroyl sarcosinate, 30% Tetramethyl-ammonium Chloride,
   <0.1% sodium azide

5. **HPV bDNA πCode MicroDisc**
   Ref. No.: 20074
   Quantity & Volume: 1 vial, 1.92 mL/vial
   Description: Control πCode MicroDisc and πCode MicroDiscs conjugated with detection probes
   Contents: Glycerol,
   Phosphate buffered saline
   0.1% Albumin, from bovine
   <0.1% EDTA
   <0.1% Sodium azide

6. **HPV bDNA POS Control**
   Ref. No.: 20077
   Quantity & Volume: 4 vials, Dried; 1 rxn/vial
   Description: Assay positive control
   Contents: Contains RNA from HEK293 cell spiked with artificially produced E6/E7 mRNA of HPV Type 18

7. **HPV bDNA Proteinase K**
   Ref. No.: 20076
   Quantity & Volume: 1 vial, 960 µL /vial
   Description: RNase inhibition
   Contents: 10-500 U/ml Proteinase K,
   <5 Mm EGTA, 0-50 mM Tris,
   <50 mM Glutathion, <50% Glycerol

   
   R36/37/38- Irritating to eyes, respiratory system and skin. R42- May cause sensitization by inhalation

8. **HPV bDNA SA-PE**
   Ref. No.: 20073
   Quantity & Volume: 1 bottle, 4.8 mL/bottle
   Description: Streptavidin-phycoerythrin for fluorescent signal acquisition
   Contents: Phosphate buffered saline
   0.5% Streptavidin-phycoerythrin
   1% Albumin, from bovine
   <0.1% Sodium azide

9. **HPV bDNA 10X Wash Buffer**
   Ref. No.: 20305
   Quantity & Volume: 2 bottles, 50 mL/bottle
   Description: For πCode washing
   Contents: Phosphate buffered saline
   1% Tween-20
   <0.1% Sodium azide

10. **HPV bDNA Sample Collection Buffer**
    Ref. No.: 20160
    Quantity & Volume: 1 vial, 200 µL/vial
    Description: Buffer solution as NEG Control and used as reconstitute buffer for POS Control.
    Contents: Methanol-Water solution

NOTE: POS Control, NEG Control and WR stand for positive control, negative control and working reagent, respectively.

8. MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- 96-well plate (Greiner Bio-one; Cat. No. 655101)
- Aluminum Micro Plate sealing tape (3M REF 9792)
- 96 well Adhesive Seal (Basic Life; Cat. No. UTI2100)
- Disposable gloves, powder-less
- Nuclease-free micro-centrifuge tubes
- Dedicated micropipette*
- Filter tips for micropipette*
- ddH_2O for dilution of 10X Wash Buffer
- Hologic® ThinPrep® Pap Test (PreservCyt® Solution) (Hologic Ref. 70097-005)
- Vortex mixer
- Microcentrifuge
- Thermo Shaker (PlexBio; Cat. No. 80011)

- IntelliPlex 1000 πCode Processor (PlexBio; Cat. No. 80033)
- PlexBio 100 Fluorescent Analyzer (PlexBio; Cat. No. 80000)
- Industrial Computer (PlexBio; Cat. No. 80002)
- DeXipher™ MD (PlexBio; Cat. No. 80051)

* Pipettes should be accurate within 3% of stated volume. Aerosol barrier or positive displacement nucleic acid- and nuclease-free tips must be used where specified to prevent cross-contamination.

9. STORAGE, STABILITY AND TRANSPORTATION

Storage

All kit components of the IntelliPlex HPV bDNA Genotyping Kit should be stored at 2°C to 8°C. Once opened, the reagent components are stable for 14 months or until the expiration date, whichever comes first.

Stability

Do not use the IntelliPlex HPV bDNA Genotyping Kit when it is expired. All components are stable up to the expiration date on the label if handled and stored under the recommended conditions.

Transportation

The shipping temperature for the IntelliPlex HPV bDNA Genotyping Kit is at 2-8°C. If the kit is broken or has components missing, please contact the PlexBio Customer Service.

10. INSTRUMENT AND SOFTWARE

Instrument

Refer to the instrument user manual for complete installation and operation instructions (Thermo Shaker, IntelliPlex 1000 πCode Processor and PlexBio 100 Fluorescent Analyzer).

Software Installation

NOTE:

1. For the first time assay operation, please make sure the KIT APP is installed into DeXipher.

2. The barcoded ENC file contains the information of kit lot no. and expiration date.

KIT APP Installation

1. Open the HPV bDNA KIT APP from the USB drive provided and run “Installer.exe”.
2. Click on “Install” from the pop up window and finish the APP installation.

Note: The KIT APP only needs to be installed for the first time running until the new version release.

ENC File Installation

1. Download the corresponding ENC File from PlexBio’s website (Homepage/ Download/ ENC Files/ HPV bDNA Kit/ Corresponding Kit Lot no.)
2. Save to the computer and make sure the ENC file matches the lot no. of the assay kit.
3. Click on the button as shown below on the DeXipher homepage.

4. Click on the button as shown below to import kit.

5. Select and import the corresponding ENC file into the software.

11. SPECIMENS

Specimen Collection

The collection of cervical specimens are only validated by Hologic® ThinPrep® Pap Test (PreservCyt® Solution).

Specimen Transportation and Storage

The shipping and storage conditions as specified in the Hologic® ThinPrep® Pap Test (PreservCyt® Solution) should be strictly followed. Cervical specimens can be stored and transported at 2-8°C for up to 3 days.

12. ASSAY PROCEDURE

Warning:

Read the instructions carefully and follow every step of the assay protocol correctly.

Note:

The bDNA test is very sensitive to temperature change. Once the plate is removed from the Thermo Shaker, the plate should be processed immediately according to the procedure.

12.1 Buffer Preparation

1. If needed, pre-warm WR, A1, A2 and A3 buffer at 37˚C for 20 minutes or until the visible crystals dissolve and mix evenly prior to use.
2. Transfer 50 mL 10X Wash Buffer to 1L Wash Buffer bottle of the IntelliPlex 1000 πCode Processor supplied and add 450 ml ddH2O to prepare 1X Wash Buffer for use.

12.2 Sample Preparation

1. Vortex specimen container for 10 seconds.
2. Remove the brush from specimen container.
3. Stand the specimen container for at least 20 minutes at room temperature.

***Note:**

- *Please make sure the cell pellet settle down at the bottom of the specimen container.*

12.3 POS Control Preparation

1. Reconstitute the POS control with 25 µL sample collection buffer. Stand for at least 3 minutes followed by pipetting mix.
2. Spin down the POS control prior to use.

***Note:**

- *POS Control is **single use after the reconstitution**. Reconstitute the POS Control every time prior to use.*
- *POS Control and NEG Control reactions should be included in every run of the assay.*
- *The Sample Collection Buffer provided in the kit is also used as NEG Control.*

12.4 Cell Lysis and First Hybridization

1. Dispense the following components, in the order indicated below, into each well of a 96 well plate: 20 µL HPV bDNA πCode MicroDisc (make sure vortex evenly), 95 µL WR and 10 µL Proteinase K.

***Note:**

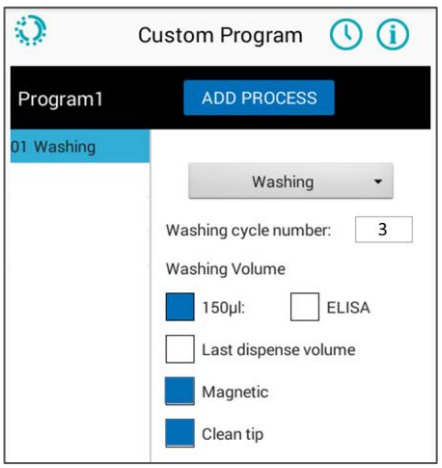
- *The sample display order on the 96 well plate is recommended from A to H.*
- *Please make sure the πCode MicroDisc vortex evenly before dispensing into wells accordingly.*

2. Add 20 µL of the settled cell pellet at the bottom of the sample container and pipetting 20 µL of the POS Control and 20 µL of sample collection buffer as NEG control into each wells, respectively.
3. Seal the 96-well plate with aluminum micro plate tape.
4. Place the 96-well plate on the Thermo Shaker and incubate at 60°C by using the program for “800 rpm for 1 hour”.
5. Lower the setting temperature to 53°C and continue to incubate at 800 rpm on the Thermo Shaker overnight*.

***Note:**

- *No more than 18 hours of first hybridization is recommended.*

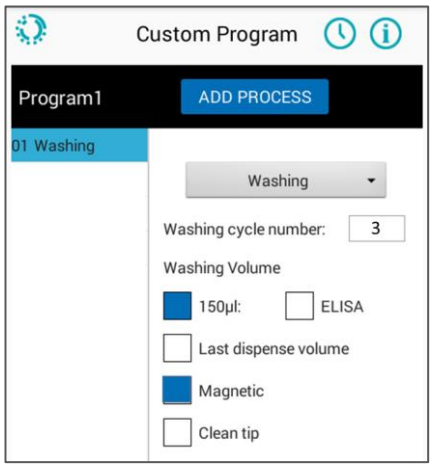
6. Remove the aluminum micro-plate tape on the Thermo Shaker at 53°C and place the plate on the IntelliPlex 1000 πCode Processor without any delay.
7. Select Custom Program and the desired rows on the IntelliPlex 1000 πCode Processor touch panel to set up the following washing conditions as figure shown below.



***Note:** Please refer to the user manual of IntelliPlex 1000 πCode Processor for all necessary operation information.

12.5 Second Hybridization (Pre-Amplifier)

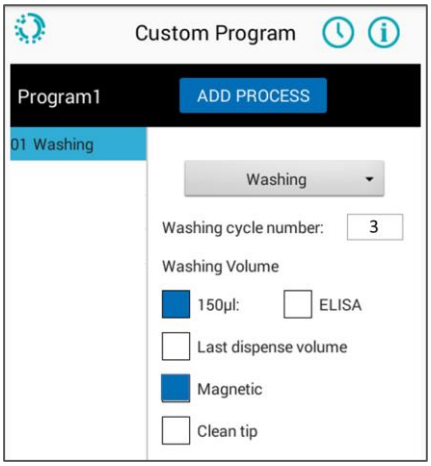
1. Dispense 50 µL HPV A1 Buffer into each well and seal the plate with 96 well Adhesive Seal.
2. Place the 96-well plate on the Thermo Shaker and incubate at 50°C, 1200 rpm for 1 hour.
3. Select Custom Program and the desired rows on the IntelliPlex 1000 πCode Processor touch panel to set up the following washing conditions as figure shown below.



12.6 Third Hybridization (Amplifier)

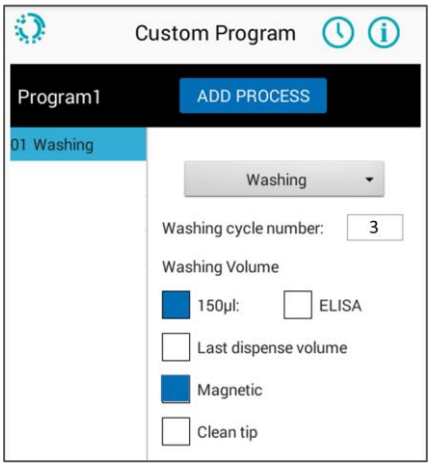
1. Dispense 50 µL HPV A2 Buffer into each well and seal the plate with 96 well Adhesive Seal.

2. Place the 96-well plate on the Thermo Shaker and incubate at 50°C, 1200 rpm for 1 hour.
3. Select Custom Program and the desired rows on the IntelliPlex 1000 πCode Processor touch panel to set up the following washing conditions as figure shown below.



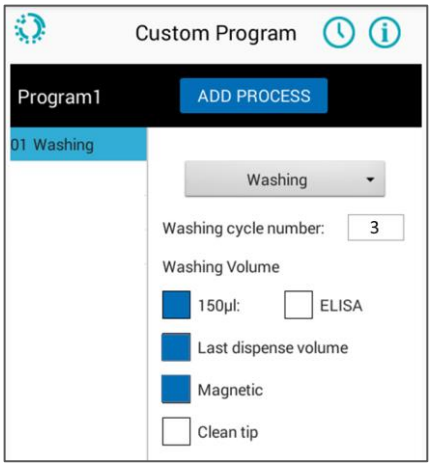
12.7 Fourth Hybridization (Labeling Probe)

1. Dispense 50 µL HPV A3 Buffer into each well and seal the plate with 96 well Adhesive Seal.
2. Place the 96-well plate on the Thermo Shaker and incubate at 50°C, 1200 rpm for 1 hour.
3. Select Custom Program and the desired rows on the IntelliPlex 1000 πCode Processor touch panel to set up the following washing conditions as figure shown below.



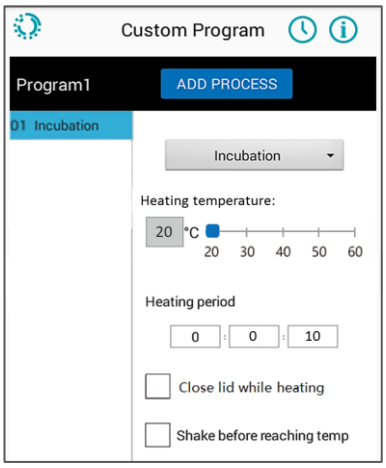
12.8 SA-PE Incubation

1. Add 50 µL of SA-PE Solution to each well and incubate at 37˚C on the Thermo Shaker by using the program for “1,200 rpm for 10 minutes”.
2. Select Custom Program and the desired rows on the IntelliPlex 1000 πCode Processor touch panel to set up the following washing conditions as figure shown below.

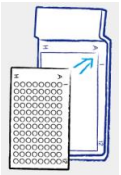


12.9 Image Decoding and Fluorescent Detection

1. Select Custom Program and the desired rows on the IntelliPlex 1000 πCode Processor touch panel to set up the shaking conditions as figure shown below.



2. Place the plate on the PlexBio 100 Fluorescent Analyzer as shown below.



3. Click on the button as shown below on the DeXipher homepage.

4. Select the well for detection and import the needed information of each detection samples. Click next step as figure shown as below.

5. Enter the “Assay Name” for the assay and click icon as shown below for image pattern decoding and fluorescent signal acquisition.

6. Export the results for data analysis or calculation or the pdf report.

13. DISCLAIMERS

Negative test result

A negative test result means the HPV RNA was not detected by the IntelliPlex HPV bDNA Genotyping kit. It does not preclude an infection with HPV types 16, 18, and other 12 high-risk genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). Moreover, false negative test results may be due to experimental errors or other causes.

Positive test result

A positive test result means the HPV RNA with types 16, 18, and other 12 high-risk genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) was detected by IntelliPlex HPV bDNA Genotyping kit. It does not preclude the possibility that the specimen did in fact not contain HPV RNA. False positive test results may be caused by experimental errors or other causes.

14. INTERPRETATION OF RESULTS

Table 2: Result Interpretation

Test Result	Report Result	Interpretation
HPV Detected	HPV 16, HPV 18, Others	HPV is detected with HPV type 16 or type 18 or any one of, or combination of, the following high risk types (31, 33. 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)
HPV Not Detected	None	HPV are undetectable
Invalid Assay	Invalid	Possible Cause: 1. SAPE Reaction Failed 2. Low RNA input or RNA degradation 3. Low πCode Disc Count 4. No πCode Detected 5. Blank πCode Control Failed

15. TROUBLESHOOTING

The troubleshooting guide listed below addresses possible problem causes and solutions provided during assay procedures.

Table 3: Troubleshooting Guide

Problem	Possible Cause	Recommendations
POS Control Fail	1. Incomplete dissolved of POS Control pellet during the reconstitution step	1. Make sure the POS Control pellet is dissolved completely
	2. POS Control RNA degradation	2. Make sure the dried POS Control is stored at 2 to 8°C. It should be immediately single use after the reconstitution
NEG Control Fail	1. HPV signal or Reference gene detected which may due to the contamination	1. Make sure the buffers and operation procedure without the contamination. Replace the new buffers if necessary.
Internal Control Fail	1. Insufficient cervical cells collected	1. Make sure the settled cell pellet is visible at the bottom of the specimen container
	2. Sample RNA degradation	2. Make sure the specimen storage condition is correct

SA-PE Monitor Control Fail	1. SA-PE solution dispensing error 2. SAPE solution inactivation	1. Make sure the sufficient SA-PE solution used during the assay operation 2. Make sure the correct storage condition of SA-PE.
Low πCode Disc Count	1. Overlapping of πCode 2. πCode Discs are not distribute evenly at each well	1. Re-suspend πCode Discs by shaking the plate for 10 seconds and decoding again 2. Make sure the πCode MicroDisc vortex evenly before dispensing into wells accordingly.

16. PERFORMANCE

Limit of Detection

The limit of detection of 14 high risk HPV genotypes were accessed by testing the artificial 14 genotypes of HPV E6/E7 RNA spiked into the HOLOGIC ThinPrep® PreservCyt® Solution. Each type of E6/E7 RNA was further serially diluted at various concentrations with total RNA derived from HEK293 cell line (HPV negative). 21 replicates of each copy level were tested using two reagent lots across 3 days for a total of 42 replicates. The limit of detection of each genotype was determined based on a positive hit rate at 95% in PriProbit analysis as shown in the table below.

Table 4: Summary of Limit of Detection Tested

Target	Limit of Detection* (95% CI)
HPV 16	1.51E+04
HPV 18	3.45E+03
HPV 31	4.48E+04
HPV 33	6.19E+04
HPV 35	4.19E+05
HPV 39	4.48E+04
HPV 45	7.38E+03
HPV 51	3.02E+04
HPV 52	3.93E+04
HPV 56	3.04E+04
HPV 58	5.62E+04
HPV 59	5.48E+04
HPV 66	4.43E+04
HPV 68	4.81E+04

*Copies per reaction

Analytical Specificity

A panel of microorganism that includes those commonly found in the female urogenital tract and HPV genotypes classified as low risk were tested to evaluate the analytical specificity. The tested inactivated bacteria, viruses or nucleic acid of microorganism with high titers were spiked into HPV negative samples (HEK293) in PreservCyt Solution or into PreservCyt Solution with HPV type 18 infected Hela cells (4,000 cells per reaction). Results indicated that the IntelliPlex HPV bDNA Genotyping Kit was not affected by any of the organisms tested.

Table 5: Microorganism Tested for Analytical Specificity

Microorganism	Test Concentration with No Cross-Reactivity
Human immunodeficiency virus 1 (HIV-1)	1000 IU/test
Epstein-Barr virus (EBV)	2000 IU/test
Herpes simplex virus 1 (HSV-1)	1000 IU/test
Herpes simplex virus 2 (HSV-2)	1000 IU/test
Cytomegalovirus (CMV)	2000 IU/test
Neisseria gonorrhoeae	1000 IU/test
Chlamydia trachomatis	1000 IU/test
Staphylococcus epidermidis	20ng/test
Bacteroides fragilis genomic DNA	20ng/test
Proteus vulgaris genomic DNA	20ng/test

Table 6: Non-targeted HPV Genotypes Tested for Analytical Specificity

Non-targeted HPV Genotypes	Test Concentration with No Cross-Reactivity
HPV 6	1 x 10^6 copies/rxn
HPV 11	1 x 10^6 copies/rxn
HPV 40	1 x 10^6 copies/rxn
HPV 42	1 x 10^6 copies/rxn
HPV 43	1 x 10^6 copies/rxn
HPV 44	1 x 10^6 copies/rxn

Interference

The interference test is designed to access the analytical specificity by testing the impact of potentially interfering substances (List as table below). The substances were spiked at highest concentration into HPV genotype 18-infected positive Hela cells and into HPV negative HEK293 cells in PreservCyt Solution. Each sample was tested in four replicates at least. The result indicated the one interference substance, Kanezin Vaginal Tablets 100MG "SWISS" (CLOTRIMAZOLE), will interfere the performance with false positive result of HPV genotype 31. In addition, the presence of whole blood will interfere the fluorescence signal value but not necessary meant to interfere the result of genotype diagnosed. Rest potentially interfering substances will not interfere with the performance of the Intelliplex HPV bDNA Genotyping Kit.

Table 7: Interfering Substances Tested

Interfering Substance	Highest Concentration Tested
Lubricating Gel	10% v/v
Personal Lubricating Jelly	10% v/v
Whole Blood	10% v/v
Statin Vaginal Tablets 100000 Units "Standard" (NYSTATIN)	50,000 unit/ml
Fortin Suppositories	12.5 mg/ml
Kanezin Vaginal Tablets 100MG "SWISS" (CLOTRIMAZOLE)	5 mg/ml
Jslady Spray Solution 3MG/ML (POVIDONE-IODINE)	10% v/v
Feminine Cleansing Mist Sensitive Skin	10% v/v
Feminine Cleansing Mist	10% v/v

Repeatability and Reproducibility

The repeatability and reproducibility of IntelliPlex HPV bDNA Genotyping Kit was evaluated with same sample panel into two studies. Reproducibility study was conducted across three reagent lots, two operators, three sets of instruments and 5 non-consecutive testing days for a total of 54 valid runs. Repeatability study was conducted across three reagent lots, two operators, three sets of instruments and 12 non-consecutive testing days for a total of 54 valid runs. The tested positive sample panel with HPV E6/E7 mRNA and HPV negative sample with HEK 293 RNA were spiked into PreservCyt Solution. Results were demonstrated with 3x LoD and 6x LoD based on the PriProbit analysis.

Table 8: Reproducibility Results at all Variances Combined

Target	Concentration	Number of Positive/ Test	Hit Rate (%)
HPV 16	3x LoD	51/54	94.4%
	6x LoD	54/54	100%
HPV 18	3x LoD	51/54	94.4%
	6x LoD	53/54	98.1%
HPV 45	3x LoD	52/54	96.3%
	6x LoD	53/54	98.1%
HPV 51	3x LoD	54/54	100%
	6x LoD	54/54	100%
HPV 56	3x LoD	53/54	98.1%
	6x LoD	54/54	100%
HPV Negative	-	0/54	100%

Table 9: Repeatability Results at all Variances Combined

Target	Concentration	Number of Positive/ Test	Hit Rate (%)
HPV 16	3x LoD	52/54	96.3%
	6x LoD	53/54	98.1%
HPV 18	3x LoD	52/54	96.3%
	6x LoD	54/54	100%
HPV 45	3x LoD	53/54	98.1%
	6x LoD	54/54	100%
HPV 51	3x LoD	54/54	100%
	6x LoD	54/54	100%
HPV 56	3x LoD	53/54	98.1%
	6x LoD	54/54	100%
HPV Negative	-	0/54	100%

Cross-Contamination

The test is designed to access the cross-contamination during all assay operation steps which may lead to the false positive results. The tested HPV negative samples (HEK293) and HPV positive samples (HPV infected Hela cells) were placed in rotated order between wells in the 96-well plate. The results demonstrated that there was no cross-contamination during all assay operation steps.

17. SYMBOLS

Symbol	Explanation	Symbol	Explanation
IVD	In-vitro diagnostic use	REF	Catalog number
LOT	Batch number		Consult instructions for use
	Manufacturer		Use by Date
	Temperature limitation		Caution
	Contains sufficient for <n> tests		Date of Manufacture
CE	European Union Conformity	EC REP	European Authorized Representative

18. REFERENCES

1. Doorbar, J. (2006). Molecular biology of human pappilomavirus infection and cervical cancer. Clin Sci (Lond). 110(5):525-41.
2. Kjaer, S.K.; van den Brule, A.J.C.; Paull, G.; Svare, E.I.; Sherman, M.E.; Thomsen, B.L.; Suntum, M.; Bock, J.E.; Poll, P.A.; Meijer, C.J.L.M. (2002). Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow up study. BMJ. 325(7364):572-579.
3. Gaarenstroom, K.N.; Melker, P.; Walboomers, J.M.M.; van den Brule, A.J.C.; Van Boomel, P.F.J.; Mejier, C.J.L.M.; Voorhorst, F.J.; Kenemans, P.; Helmerhorst, T.J.M. (1994). Human papillomavirus DNA and genotypes: prognostic factors for progression of cervical intraepithelial neoplasia, Int. J Gynecol Cancer; 4:73-78.
4. Bosch, F.X.; Lörincz, A.; Muñoz, N.; Meijer, C.J.L.M.; Shah, K.V. (2002). The causal relation between human papillomavirus and cervical cancer. J Clin Path, 55: 244-265.
5. Schlect, N.F. et al. (2001). Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA, 286:3106-3114.
6. Nobbenhuis, M.A.E.; Walboomers, J.M.M. et al. (1999). Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. Lancet, 354:20-25.
7. Castle, P.E.; Wacholder, S.; Sherman, M.E.; Schiffman, M. et al. (2002). Absolute risk of a subsequent abnormal pap among oncogenic human papillomavirus DNA-positive, cytologically negative women. 95(10):2145-2151.
8. Klug, S.; Molijn, A. et al. (2008). Comparison of the performance of different HPV genotyping methods for detecting genital HPV types. Journal of Medical Virology, 80:1264-1274.
9. International Agency for Research on Cancer (IARC) Working Group on the Evaluation of Carcinogenic Risks to Humans. Human Papillomaviruses. Lyon, France: World Health Organization; 1995. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 64.
10. Berek, J.S. Practical Gynecologic Oncology, Lippincot Wiliams, Fourth Edition.
11. Pfister H. Biology and biochemistry of papillomaviruses Rev Physiol Biochem Pharmacol 1983.
12. Monsonego, J.; Bosch, F.X.; Coursaget, P.; Cox, J.T.; Franco, E.; Frazer, I.; Sankaranarayanan, R.; Schiller, J.; Singer, A.; Write Jr., T.C.; Kinner, W.; Meijer, C.J.; Linder, J.; McGoogan, E.; Meijer, C. (2004). Cervical cancer control, priorities and new directions. Int J cancer. 108(2): 329-333. Erratum in: Int J Cancer. 108(6): 945.
13. Baseman, J.G.; Koutsky, L.A. (2005). The epidemiology of human papillomavirus infections. J Clin Virol. 32 Suppl1:S16-24.
14. Czegledy, J.; Losif, C.; Hansson, B.G.; Evander, M.; Gergely, L.; Wadell, G. (1995). Can a test for E6/E7 transcripts of human papillomavirus type 16 serve as a diagnostic tool for the detection of micrometastasis in cervical cancer? Int J Cancer. 64(3):211-215.

Notice to User

The use of this product and the associated PlexBio instrumentation is covered by one or more issued (US 9,063,044B2) and pending US and foreign patents owned by PlexBio Co., Ltd. The purchase of this product includes nontransferable rights to use this amount of the product to practice the methods described therein. No general patent or other license of any kind other than this specific right of use from purchase is granted. For further information on purchasing licenses for other applications can be obtained from PlexBio Co., Ltd. 6F-1, No. 351, Yangguang St., Neihu District, Taipei City 11491, Taiwan. The bDNA technology in this product is provided under an agreement between DiaCarta, Ltd. and PlexBio Co., Ltd., and is covered by patent rights licensed by DiaCarta, Ltd.

Trademarks

Copyright

PlexBio Co., Ltd.
6F-1 No. 351 Yangguang St.,Neihu District, Taipei 11491
Tel. | +886-2-2627-5878 **Email |**contact@plexbio.com

DiaCarta, Inc.
2600 Hilltop Drive, Richmond, CA 94806
Tel. | 1-800-246-8878 **Email |** information@diacarta.com