

INSTRUCTIONS FOR USE

NeoPlex™ HPV HR

REF

NS03A

NeoPlex™ HPV HR Detection

Multiplex Real-time PCR Reagents for Human papilloma virus detection
For professional *in vitro* diagnostic use only



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NeoPlex™ HPV HR

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1. INTENDED USE

The 'NeoPlex™ HPV HR' is a qualitative *in vitro* test for the simultaneous detection and confirmation of cervical cancer-causing pathogens HPV(human papillomavirus) 14 genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) from liquid-based cytology specimen. This test kit is intended for professional use.

2. KIT CONTENTS

The 'NeoPlex™ HPV HR' components are shown in the table below.

Contents	Volume(96T)	Storage condition	Shelf life
4X NeoPlex PCR Master Mix	500 µL x 1 Vial		12 months (Before opening)
HPV HR PPM	500 µL x 1 Vial		
HPV HR Positive Control(PC)	100 µL x 1 Vial	Upper limit -20°C	6 months (After opening)
HPV HR Internal Control(IC)	1 mL x 1 Vial		
DW(DNase-free Water)	1 mL x 1 Vial		

3. COMPATIBLE INSTRUMENT

- CFX96™ Dx System (Bio-Rad, Cat No.1845097-IVD)

4. NUCLEIC ACID EXTRACTION

Manufacturer	Instrument (Cat No.)	Extraction Kit (Cat No.)
Qiagen	N/A (Manual)	QIAamp DSP DNA Blood Minikit (61104)
Roche Life Science	Roche MagNA Pure 96 system (06 541 089 001)	DNA and Viral NA Small Volume Kit (06543588001)
Hanwool TPC	NC-15 PLUS (HWTD-01-48)	AlphaPrep™ Viral DNA/RNA Extraction Kit (VDR-B096V)

5. ADDITIONAL REQUIRED EQUIPMENT & MATERIALS

- 0.2 ml 8-Tube PCR Strips without Caps, low profile, white (Bio-Rad, Inc., Cat No. TLS0851)
- Optical Flat 8-Cap Strips for PCR Tubes (Bio-Rad, Inc., Cat No. TCS0803)
- Pipettes set, P2/P10, P20, P200, and P1000 aerosol barrier tips
- Micro Centrifuge
- Vortexer mixer
- Disposable powder-free gloves



Use PCR plate strip caps only. Do not use PCR plate sealing film.

6. KIT STORAGE AND STABILITY

- Store the kit below -20°C.
- Kit materials are stable until the expiration date printed on the label under un-opened condition.
- Kit's shelf life is twelve (12) months.
- Please use the reagents within six (6) months after opening.

7. WARNINGS AND PRECAUTIONS

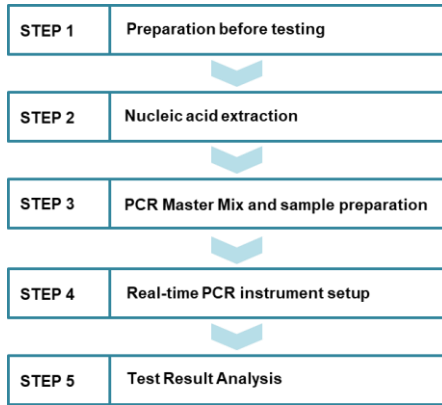
1. This device is intended for *in vitro* use only. Do not use the device for other purposes.
2. Wear personal protective equipment, such as gloves and lab coats when handling NeoPlex™ HPV HR and/or specimens.
3. Do not smoke, drink, or eat while handling NeoPlex™ HPV HR and/or samples.
4. Please be careful when handling samples to prevent infections of user and/or indirect contact to a person. Sample contains a risk of infections and unknown diseases.
5. Do not use reagents from different lots or from different tubes of the same lot.
6. If you do not frequently inspect the product, keep a kit in a refrigerator for a certain amount of time. Do not freeze/thaw over four times. Repeated frozen/thawed product may result in false negative and false positive results.
7. Be careful not to contaminate the product when extracting nucleic acid, amplifying PCR product, using positive control(PC).
8. The use of filter tips is recommended to prevent contamination of the product.
9. It is recommended that the sample or the positive control(PC) contained in the product to be frozen and stored separately from the freezer storing the product.
10. Use the sterilized consumable laboratory supplies. Do not reuse it.
11. Add the extracted nucleic acid sample and positive control(PC, Positive Control) into the reaction solution in a space separate from the PCR reaction solution preparation space.
12. Before using, read this instruction for use carefully.
13. Use calibrated measuring tools. (e.g. pipette)
14. Please check the expiration date before using the reagent.
15. Keep Positive Control(PC) separately when using to avoid contamination.
16. Before starting the PCR, make sure the lid is closed properly.
17. Dispose the product in accordance with local or national regulations.
18. Please consult with doctor about the test results.

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7. TEST PROCEDURE



STEP 1. Preparation before testing

1) Preparation before testing

- Prepare all the devices and reagent before use.
- Place the kit on ice when thawing components and preparing PCR Master Mix.
- After preparing PCR master mix, place them on ice.



Do not freeze/thaw over four (4) times.

2) Specimen Collection, Transportation and Storage

- Specimens for use: Liquid based cytology specimen.
- Store specimens at 2~8 °C for no longer than 4 weeks. For pro-longed storage, Freeze under -20 °C condition.
- Extracted nucleic acids should be stored at -20 °C or lower.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.



- Use only the specimen type listed in the instruction manual.
- Wear eye protection, laboratory coats and disposable gloves when handling specimens.
- Specimens should be stored under the storage conditions above. Otherwise, the wrong test results can be obtained.
- Sample information should be recorded to avoid confusion.

STEP 2. Nucleic acid extraction

After pre-treatment, extract nucleic acid from the sample. Nucleic acid extraction can be done by automated purification system or using manual prep kits.

We recommend following the table at the bottom nucleic acid extraction kit/automatic machine for nucleic acid extraction. Also, for nucleic acid extraction, follow the manufacturer's protocol.

Instrument (Cat No.)	Reagent (Cat No.)	Manufacturer	Elution volume	Sample volume
N/A (Manual)	QIAamp DSP DNA Blood Mini Kit (61104)	Qiagen	80 µL	200 µL
MagNA Pure 96 system	MagNA Pure 96 DNA and Viral Small Volume Kit AlphaPrep™ Viral	Roche Life Science	50 µL	200 µL
NC-15 PLUS (HWTD-01-48)	DNA/RNA Extraction Kit (VDR-B096V)	Hanwool TPC	50 µL	200 µL

STEP 3. PCR Master Mix and sample preparation

1) Prepare the Master Mix

① PCR Mixture

Contents	Volume (1 test)
4X NeoPlex PCR Master Mix	5 µL
HPV HR PPM	5 µL
DW(DNase-free Water)	5 µL
Total Volume	15 µL

Note: Calculate the required amount of each reagent based on the number of reactions (samples + controls).

- Mix by inversion 5 times or by vortexing and centrifuging briefly.
- Place 15 µL aliquots of the PCR Master mix into 0.2 ml PCR tubes and close the lids.
- Add 5 µL of each nucleic acid sample to its respective tube.

Contents	Volume (1 test)
PCR Mixture	15 µL
Nucleic acid sample	5 µL
Total Reaction Volume	20 µL



- It is recommended that the PCR mixture to be prepared just before use.
- Aerosol-resistant filter tips and tight gloves should be used when preparing samples.
- Take great care to avoid cross contamination.
- Defrost the reagents completely.
- Centrifuge the reagent tubes briefly to remove the drops from the inside of the lids.

5) Make the control amplification reactions.

- Negative Control (NC): Add 5 µL of DW(DNase-free Water) instead of nucleic acid samples to the tube.
- Positive Control (PC): Add 5 µL of HPV HR Positive Control(PC) instead of nucleic acid samples to the tube.



- Use a new pipette tip with each different sample.
- Avoid cross-contamination of PCR Master Mix and samples with Positive Control.
- Do not label on the cap of the reaction tubes as fluorescence is detected through the cap.
- Centrifuge the PCR tube thoroughly for 30 seconds.

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STEP 4. Real-time PCR instrument setup

1) Selection of fluorescence channels.

Target	Dye	Target	Dye
HPV 33	FAM	HPV 39	Quasar 670
HPV 16	FAM	HPV 56	Quasar 670
HPV 35	FAM	HPV 52	Quasar 670
HPV 18	HEX	HPV 45	Quasar 705
HPV 66	HEX	HPV 59	Quasar 705
HPV 68	Cal Red 610	HPV 51	Quasar 705
HPV 58	Cal Red 610	IC	HEX
HPV 31	Cal Red 610	-	-

2) Setting the PCR protocol.

PCR protocol should be set according to the table as below.

Segment	Tm(°C)	Time	Cycles
1	50	4 min	1
2	95	15 min	1
3	95	30 sec	40
4	65	75 sec	40
5	73	10 min	1
6	55	30 sec	1
7*	Melting curve 55 °C ~ 91 °C (5s/0.5°C)		

* Segment 7: Melting curve measurement

STEP 5. Test result analysis

For the analysis of the test result after PCR amplification, take the RFU value result and interpret the according to '9. INTERPRETATION OF TEST RESULTS'.

9. INTERPRETATION OF TEST RESULTS

For the analysis of the test result after PCR amplification, take the melting peak result (For CFX96 check the 'Melt Peak' tab) and interpret the according to the following interpretation table.

1. Interpretation criteria for result analysis

Target	Dye	Melt Tm	Cut-off(RFU*)
HPV 33	FAM	65.0 ± 1.5°C	≥ 93.5
HPV 16	FAM	73.0 ± 1.5°C	≥ 98.3
HPV 35	FAM	80.5 ± 1.5°C	≥ 93.9
HPV 18	HEX	75.0 ± 1.5°C	≥ 95.1
HPV 66	HEX	83.5 ± 1.5°C	≥ 98.2
HPV 68	Cal Red 610	64.0 ± 1.5°C	≥ 93.0
HPV 58	Cal Red 610	71.5 ± 1.5°C	≥ 91.5
HPV 31	Cal Red 610	77.5 ± 1.5°C	≥ 94.5
HPV 39	Quasar 670	64.5 ± 1.5°C	≥ 99.7
HPV 56	Quasar 670	73.0 ± 1.5°C	≥ 97.8
HPV 52	Quasar 670	79.0 ± 1.5°C	≥ 97.3
HPV 45	Quasar 705	66.0 ± 1.5°C	≥ 92.0

HPV 59	Quasar 705	73.5 ± 1.5°C	≥ 95.1
HPV 51	Quasar 705	80.5 ± 1.5°C	≥ 98.7
IC	HEX	66.0 ± 1.5°C	≥ 100

* RFU(-d(RFU)/dT): Relative fluorescence units

2. Interpretation of result

Target	IC	Result	Result
+	+	Detected	Target is detected.
-	+	Not detected	Target is not detected.
-	-	Not detected (IC Invalid)	<ul style="list-style-type: none"> Negative(-) result of IC is the result of inhibition of PCR reaction due to the presence of a PCR inhibitor contained in the sample, and the ample is not suitable for the test. It is recommended to remove the PCR inhibitor and perform the nucleic acid extraction again. If the IC is not detected after the retest, To verify the nucleic acid extraction process and the presence of PCR inhibitors, the experimental process can be verified by adding 10 ul of HPV HR Internal Control (IC) contained in the product to the sample and then extracting nucleic acid.
+	-	Detected	<ul style="list-style-type: none"> If the nucleic acid concentration is high in the sample, the IC signal may be attenuated. If a target pathogen is detected in the sample and no IC is detected, the target pathogen is determined to be detected. if you want to check IC, dilute the template nucleic acid in distilled water and repeat the PCR with the diluted nucleic acid.

* IC is not necessary for the interpretation of positive or negative results and high load of pathogen's nucleic acid results in the low signal or negative signal of IC.

* If PC are identified above its cut-off, it may indicate that the kit has been handled in an improper way and should be replaced with a new one.

3. Application examples of clinical samples

No	Target													Result		
	33	16	35	18	66	68	58	31	39	56	52	45	59		51	IC
Sample 1	-	+	-	-	-	+	-	-	-	-	-	+	-	-	+	HPV 16, 68, 45
Sample 2	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	HPV 18, 39
Sample 3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	HPV 33
Sample 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	Not detected
Sample 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Not detected (IC Invalid)
Positive Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Positive Control
Negative Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Negative Control

4. Precaution for result analysis

- 1) If the Positive Control(HPV HR Positive Control(PC)) and RFU(-d(RFU)/dT) value is outside the acceptable range, invalidate all relevant tests and retested.
- 2) If amplification is observed in the Negative Control(NC), it is determined that contamination occurred during the experiment and a retest is conducted. However, if the same result is confirmed after the retest, it is recommended to remove the source of contamination and perform the nucleic acid extraction process again.
- 3) Internal Control(IC) should always be amplified, and if the

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concentration of nucleic acid in the sample is high, the IC signal may be impaired, resulting in a reduction or offset of the signal. If you want to check the IC, it is recommended to dilute the sample and retest it.

- If both the target and IC are determined to be negative, the presence of inhibitors within the extracted nucleic acid hindered the PCR reaction. It is recommended that the nucleic acid extraction process be carried out again.

10. QUALITY CONTROL

NeoPlex™ HPV HR includes HPV HR Positive Control(PC) as Positive Control and DW(DNase-free Water) as Negative Control. For all runs, valid test results must be obtained for PC and NC. Positive Control result must be Positive (Valid). Negative Control result must be Negative (Valid). If the positive and negative control results are consistently invalid, contact us for technical assistance.

11. TROUBLE SHOOTING

1. If all signals are not detected.

Potential causes	Solution
Error in specimen collection	If target and IC, PC signal were not observed, recollect the specimen
Nucleic acid extraction failure	Carefully read the instruction for use of nucleic acid extraction kit and extract the nucleic acid from specimen again.
Incorrect PCR setting	Repeat the detection procedure with a correct setting
Incorrect PCR cycle or machine temperature	Check the PCR conditions and repeat the PCR under the correct setting if necessary
The fluorescence for data analysis does not comply with the protocol	Select the correct fluorescence for each target listed in this Instruction guide for data analysis
Leaving reagents at room temperature for a long time or incorrect storage condition	Check the storage conditions and the expiration date of the reagents and use a new kit
Using frozen/thawed over four(4) times reagent	Do not freeze and thaw repeatedly exceed four(4) times. Because sensitivity of the reagent may decrease. Check the Freezing/thawing times and use a new kit
Presence of inhibitor	Dilute the template nucleic acid in distilled water (10-100x) and repeat the PCR with the diluted nucleic acid (If specimen is still present, restart from nucleic acid extraction procedure)
High load of pathogen's nucleic acid	Dilute the template nucleic acid in distilled water (10-100x) and repeat the PCR with the diluted nucleic acid

2. If the Negative Control signal is detected/False positive.

Potential causes	Solution
Presence of cross contamination	Decontaminate all surfaces and instruments with sodium hypochlorite or ethanol. Use filter tips during the extraction procedure. Change tips among tubes. Repeat the nucleic acid extraction with the new set of reagents

3. If the Positive control signals is Negative/False negative.

Potential causes	Solution
Error in specimen collection	Recollect the specimen and repeat the whole process. Make sure the product is stored in

	recommended conditions.
Error in nucleic acid extraction	Re-extract the nucleic acid
Incorrect PCR setting	Repeat the PCR with corrected setting
Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers for nucleic acid containing tubes and make sure to add nucleic acid into correct PCR tubes during detection process.
Incorrect PCR mixture	Check whether all components are added or not (If you use to pre-composed premix, should be reduce sensitivity) Each reagent should be used after homogenization and spin down reagent tube before putting the real-time PCR
If PC signal is invalid	If PC is identified above the cut-off, it may indicate that the kit has been handled in improper way should be replaced with a new one.

12. PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

1.1 Limit of Detection (LoD)

This study was conducted to determine the sensitivity by testing Liquid based cytology specimen. The proportion of positive results obtained from each concentration was subjected to 95% hit rate by probit analysis, and LoD of each target were obtained by performing 24 times of the tests.

Target	LoD	Target	LoD
HPV 33	0.51 copies/ul	HPV 31	1.72 copies/ul
HPV 16	0.46 copies/ul	HPV 39	0.53 copies/ul
HPV 35	1.30 copies/ul	HPV 56	1.94 copies/ul
HPV 18	0.59 copies/ul	HPV 52	1.50 copies/ul
HPV 66	1.04 copies/ul	HPV 45	0.56 copies/ul
HPV 68	1.12 copies/ul	HPV 59	0.55 copies/ul
HPV 58	1.68 copies/ul	HPV 51	0.51 copies/ul

1.2 Cut-off value

For the cut-off establishment, RFU value was set up as shown below in the table for each target.

Target	Cut-off (RFU*)	Target	Cut-off (RFU*)
HPV 33	≥93.5	HPV 39	≥99.7
HPV 16	≥98.3	HPV 56	≥97.8
HPV 35	≥93.9	HPV 52	≥97.3
HPV 18	≥95.1	HPV 45	≥92.0
HPV 66	≥98.2	HPV 59	≥95.1
HPV 68	≥93.0	HPV 51	≥98.7
HPV 58	≥91.5	IC	≥100
HPV 31	≥94.5	-	-

* RFU(-d(RFU)/dT): Relative fluorescence units

2. Analytical Specificity

2.1 Interference

Total eight (8) substances, endogenous and exogenous source, were studied to determine their interfering effect and no interference reactions was found with the concentration as below.

No	Types	Interfering substance	Concentration
1	Endogenous substances	Blood plasma	5 %
2		Red blood cell	5 %

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3		Buffy coat	5 %
4		Human genomic DNA	1 ug
5	Exogenous substances	PBS	1 %
6		Douches	2 %
			5 %
7		Antifungal ointment	2 %
			5 %
8		Vaginal lubricants	2 %
	5 %		

2.2 Cross reactivity

For analytical specificity, three (3) times of cross reactivity study using twenty-one (21) different pathogens were performed to determine the analytical specificity. As a result, PCR amplification and cross reactivity were not observed with the below pathogens.

No	Strain No.	Strain	Concentration
1	ATCC 49145D-5	Gardnerella vaginalis	1.11X10 ⁷ copies/ul
2	ATCC 700724D-5	Haemophilus ducreyi	1.09X10 ⁷ copies/ul
3	ATCC 10231D-5	Candida albicans	1.16X10 ⁸ copies/ul
4	ATCC VR-539	Human herpesvirus 1 DNA	1X10 ⁶ copies/ul
5	ATCC VR-540	Human herpesvirus 2 DNA	1X10 ⁶ copies/ul
6	ATCC 4357D-5	Lactobacillus acidophilus	7.89X10 ⁸ copies/ul
7	ATCC 70069D-5	Streptococcus pneumoniae	4.3X10 ⁸ copies/ul
8	ATCC 700928D-5	Escherichia coli	7.14X10 ⁷ copies/ul
9	ATCC 25285D-5	Bacteroides fragilis	8.4X10 ⁷ copies/ul
10	ATCC 13047D-5	Enterobacter cloacae	6.09X10 ⁷ copies/ul
11	ATCC 12453D	Proteus mirabilis	1.14X10 ⁸ copies/ul
12	ATCC 700802D-5	Enterococcus faecalis	1.32X10 ⁸ copies/ul
13	ATCC 12228D-5	Staphylococcus epidermidis	1.41X10 ⁸ copies/ul
14	HPKTCC B3204	Neisseria meningitidis	1x10 ⁵ copies/ul
15	ATCC VR-348BD	Chlamydia trachomatis(CT)	8.89X10 ⁵ copies/ul
16	ATCC 53420D-5	Neisseria gonorrhoeae(NG)	2.15X10 ⁷ copies/ul
17	ATCC 23114D	Mycoplasma hominis(MH)	1.39X10 ⁴ copies/ul
18	ATCC 33530D	Mycoplasma genitalium(MG)	1.6X10 ⁴ copies/ul
19	ATCC 30001D	Trichomonas vaginalis(TV)	4.3X10 ⁵ copies/ul
20	ATCC 33695	Ureaplasma urealyticum(UU)	1.06X10 ⁴ copies/ul
21	ATCC 27815	Ureaplasma parvum(UP)	10 ⁸ ccu/vial

The result of cross-activity for the non-target genotypes (82, 6, 53, 61, 70, 73, 26, 67, 40, 44, 11, 42, 54, 43, 69) of NeoPlex™ HPV HR, no nonspecific signals due to the cross-HPV genotypes were observed.

2.3 Carry-over & Cross-contamination

This study was performed to evaluate the carry-over and potential cross contamination effect. High concentrated positive sample and negative control sample were cross tested using same PCR instrument, and 100% negative results (84/84) (95% CI: 95.70%-100%) for each

negative specimen were determined, respectively.

3. Precision

3.1 Repeatability

Repeatability was assessed by testing for twenty (20) different days, two (2) runs per day, three (3) cycles per run. Targets were set in three (3) levels of concentration (Low, Medium, High), and 100% agreement was found determining the repeatability. The CV criteria, 10%, was met for all test results.

3.2 Reproducibility

The reproducibility study was performed with four different conditions: for Between-lot (3 lots), Between-tester (3 testers), Between-instrument (3 instruments), and Between-site (3 sites). All results showed 100% agreements.

4. Clinical Evaluation

The clinical evaluation studies for clinical sensitivity, clinical specificity and correlation were performed with the 3802 liquid based cytology specimens. The clinical study results were analyzed with 2x2 table, and summarized as below:

4.1 Clinical Accuracy (Clinical Sensitivity & Specificity)

We concluded that the clinical sensitivity and specificity of the test reagent is valid to meet the clinical effectiveness.

Target	Clinical sensitivity	Clinical specificity
HPV 33	98.02% [95% CI: 93.03 - 99.76]	99.84% [95% CI: 99.54-99.97]
HPV 16	99.54% [95% CI: 97.47 - 99.99]	99.89% [95% CI: 99.59 - 99.99]
HPV 35	98.90% [95% CI: 94.03 - 99.97]	99.79% [95% CI: 99.46 - 99.94]
HPV 18	99.19% [95% CI: 95.59 - 99.98]	100.00% [95% CI: 99.80 - 100.00]
HPV 66	98.32% [95% CI: 94.06 - 99.80]	99.89% [95% CI: 99.61 - 99.99]
HPV 68	100.00% [95% CI: 96.58 - 100.00]	99.84% [95% CI: 99.53 - 99.97]
HPV 58	98.80% [95% CI: 95.74 - 99.85]	99.95% [95% CI: 99.69 - 100.00]
HPV 31	100.00% [95% CI: 96.61 - 100.00]	99.89% [95% CI: 99.62 - 99.99]
HPV 39	99.35% [95% CI: 96.41 - 99.98]	99.89% [95% CI: 99.61 - 99.99]
HPV 56	98.90% [95% CI: 94.03 - 99.97]	99.84% [95% CI: 99.54 - 99.97]
HPV 52	100.00% [95% CI: 98.09 - 100.00]	99.89% [95% CI: 99.60 - 99.99]
HPV 45	100.00% [95% CI: 95.32 - 100.00]	100.00% [95% CI: 99.81 - 100.00]
HPV 59	100.00% [95% CI: 95.70 - 100.00]	99.95% [95% CI: 99.71 - 100.00]
HPV 51	98.99% [95% CI: 94.50 - 99.97]	99.95% [95% CI: 99.71 - 100.00]

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4.2 The agreement between NeoPlex™ HPV HR Detection and the comparator (Correlation)

Overall agreement between the NeoPlex™ HPV HR Detection and comparator method was confirmed as below.

Target	Positive agreement	Negative agreement	Total agreement	Kappa value
HPV33	99.01% [95% CI: 94.61-99.97]	99.89% [95% CI: 99.62-99.99]	99.85% [95% CI: 99.56-99.97]	0.984
HPV16	100.00% [95% CI: 98.32-100.00]	99.94% [95% CI: 99.69-100.00]	99.95% [95% CI: 99.72-100.00]	0.997
HPV35	98.91% [95% CI: 94.09-99.97]	99.84% [95% CI: 99.54-99.97]	99.80% [95% CI: 99.49-99.95]	0.977
HPV18	100.00% [95% CI: 97.02-100.00]	99.95% [95% CI: 99.70-100.00]	99.95% [95% CI: 99.72-100.00]	0.996
HPV66	99.16% [95% CI: 95.41-99.98]	99.95% [95% CI: 99.70-100.00]	99.90% [95% CI: 99.64-99.99]	0.991
HPV68	99.07% [95% CI: 94.95-99.98]	99.89% [95% CI: 99.62-99.99]	99.85% [95% CI: 99.56-99.97]	0.985
HPV58	100.00% [95% CI: 97.76-100.00]	99.84% [95% CI: 99.52-99.97]	99.85% [95% CI: 99.56-99.97]	0.990
HPV31	100.00% [95% CI: 96.55-100.00]	99.79% [95% CI: 99.46-99.94]	99.80% [95% CI: 99.49-99.95]	0.980
HPV39	98.71% [95% CI: 95.42-99.84]	99.95% [95% CI: 99.70-100.00]	99.85% [95% CI: 99.56-99.97]	0.989
HPV56	98.92% [95% CI: 94.15-99.97]	99.95% [95% CI: 99.71-100.00]	99.90% [95% CI: 99.64-99.99]	0.989
HPV52	100.00% [95% CI: 98.10-100.00]	99.94% [95% CI: 99.69-100.00]	99.95% [95% CI: 99.72-100.00]	0.997
HPV45	100.00% [95% CI: 95.32-100.00]	100.00% [95% CI: 99.81-100.00]	100.00% [95% CI: 99.81-100.00]	1.000
HPV59	100.00% [95% CI: 95.60-100.00]	99.84% [95% CI: 99.54-99.97]	99.85% [95% CI: 99.56-99.97]	0.981
HPV51	100.00% [95% CI: 96.27-100.00]	99.89% [95% CI: 99.62-99.99]	99.90% [95% CI: 99.64-99.99]	0.989

13. LIMITATION OF TEST

- Results from this test must be correlated with the clinical history, epidemiological data, and other data of the patient available to the clinician.
- If you do not use the samples and other specimens described in this manual, you may get inaccurate results.
- Although the results of this test are negative, it is not advisable to exclude the possibility that the infection is actually present.
- It is not excluded that this kit shows false positive results due to the presence of cross-contamination.
- False negative results may occur due to polymerase inhibition. HPV HR Internal Control(IC) may help to identify any substance existing in the specimens interfering with nucleic acid isolation and PCR amplification.
- This kit is for professional use only. Only trained healthcare provider can use this kit.

14. SYMBOLS

Catalogue number	Batch code	Date of manufacture	Use-by date	Distributor
<i>In vitro</i> diagnostic medical device	Upper limit of temperature	Caution	Consult instruction for use	Importer
Manufacturer	Contains sufficient for <n> tests	Authorized representative in the European Community	Conformity to European Directive 98/79/EC	Unique Device Identification

GeneMatrix Inc.
Manufacturing site
7F, #B, Korea Bio Park, 700, Daewangpangyo-ro,
Bundang-gu, Seongnam-si, Gyeonggi-do, 13488
REPUBLIC OF KOREA
Tel: +82-31-628-2045 Fax: +82-31-628-2002

MT Promedt Consulting GmbH
Ernst-Heckel-Straße 7
66386 St. Ingbert, Germany
Tel: +49-6894-581020, Fax: +49-6894-581021



Issue date: 2022.07

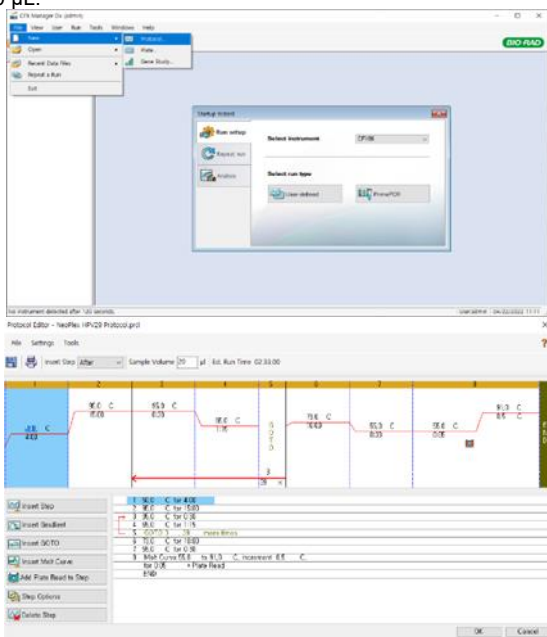
Multiplex Real-time PCR Reagents for Human papilloma virus detection For professional *in vitro* diagnostic use only

Appendix. PCR Instrument Operation

1) CFX96™ Dx System (Bio-Rad)

1. Protocol Setup

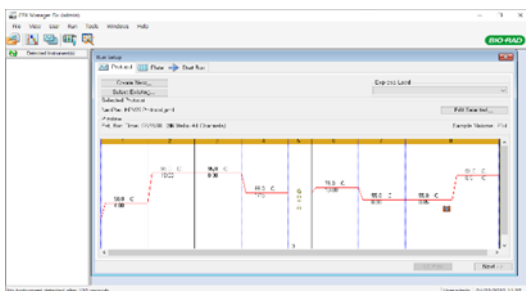
- Click File -> Protocol, Create a Protocol editor for PCR setup.
- The PCR condition is set as follows, and the sample volume set the 20 µL.



Segment	Tm(°C)	Time	Cycles
1	55	4 min	1
2	95	15 min	1
3	95	30 sec	40
4	65	75 sec	
5	73	10 min	1
6	55	30 sec	1
7*	Melting curve 55 °C ~ 90 °C (5s /0.5°C)		

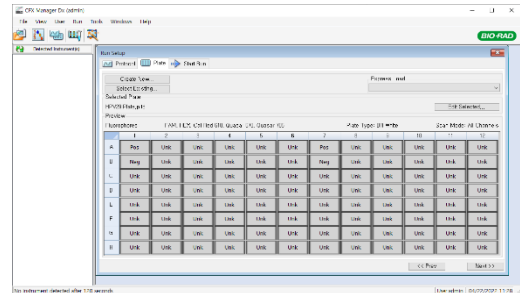
* Segment 7: Melting curve measurement

- After setting the PCR protocol, an Experiment Setup screen is created. Check the PCR protocol and click the "Next". (Or click the "Plate" tab)

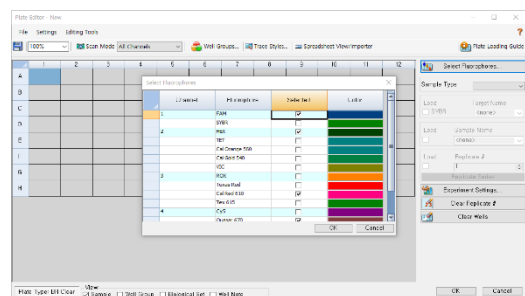


2. Protocol Setup

- Click the "Create New" (or click the "Select Existing" load and existing plate for the experiment)



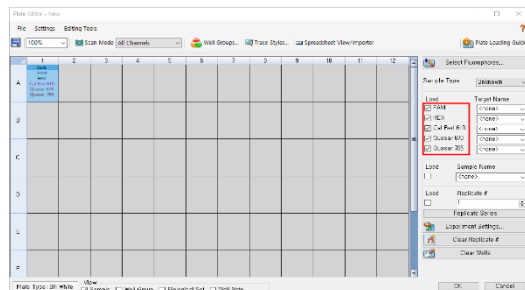
- Click the "Select Fluorophores". Select the check box (FAM, HEX, Cal Red 610, Quasar 670, Quasar 705) for the fluorescent substance used for the experiment and click the OK button.



- Select wells and select Sample Type from the drop-down menu.

Sample Type
'Unknown' : Clinical samples
'Negative control'
'Positive control'

- Click the check box for the fluorescent substance (FAM, HEX, Cal Red 610, Quasar 670, Quasar 705) of the selected well.

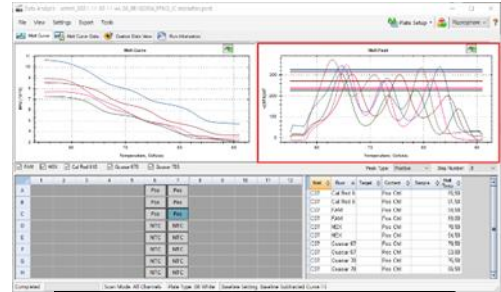


- Click the "Settings" to set the plate type. (Settings -> Plate Type -> BR white)

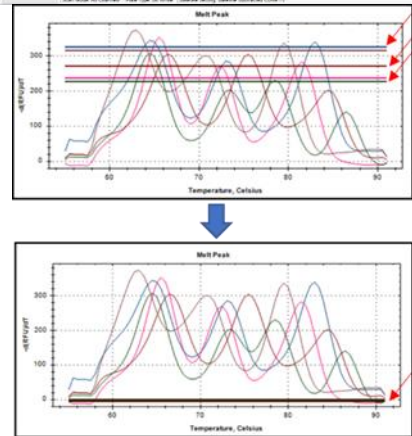
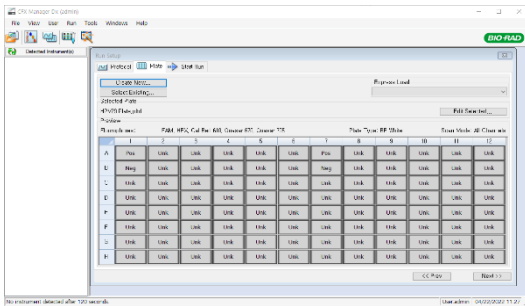
NeoPlex™ HPV HR



Multiplex Real-time PCR Reagents for Human papilloma virus detection
For professional *in vitro* diagnostic use only

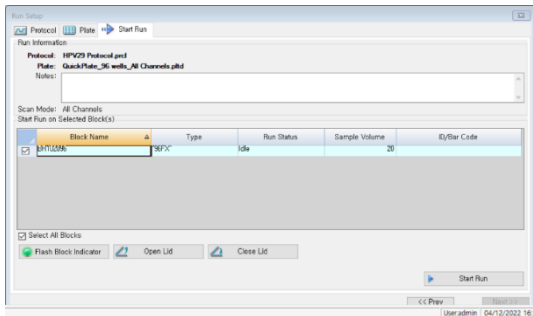


- ⑥ Click the “OK” and save a new Plate set-up file.
- ⑦ The Experimental Setup screen opens and checks the set plate. Click the “Next” (Or click the “Start Run” tab)



3. Start Run

- ① In the Experiment Setup Start Run Tab screen, click the “Close Lid” to close the lid of the equipment. (If the lid is closed, skip the step)



- ② Click the “Start Run”.
- ③ The operating file is stored in the user's designated folder, and the equipment begins to operate.

4. Pre-setting for Data analysis

- ① After the test, select the Melt curve to check the Melt Peak results.
- ② Select each analytical fluorescent substance (FAM, HEX, Cal Red 610, Quasar 670, Quasar 705) and set the threshold bar of Melt Peak to "0".