

## INNO-LiPA HPV Genotyping *Extra*



### Manufactured by:

INNOGENETICS N.V.  
Technologiepark 6  
9052 Gent  
Belgium  
☎+32-9 329 13 29  
BTW BE 0427.550.660  
RPR Gent

“Note changes highlighted”

### Distributed by:

INNOGENETICS GmbH  
Hans-Böckler-Allee 20  
30173 Hannover  
Germany  
☎+ 49-511-8573931

INNOGENETICS s.a.r.l.  
Les Conquérants, Bât. Le Kilimandjaro  
8/10, avenue des Tropiques  
91940 Les Ulis  
France  
☎+33-1 69 07 48 34

INNOGENETICS S.r.l.  
Via Vaccareccia 39/A  
00040 Pomezia (Roma)  
Italy  
☎+39-06 965 28 700

INNOGENETICS Diagnóstica Iberia, S.L.U.  
Calle Tarragona 161, Planta 14  
08014 Barcelona  
Spain  
☎+34-93 270 53 00

INNOGENETICS N.V.  
Technologiepark 6  
9052 Gent  
Belgium  
☎+32-9 329 13 29

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<b>GR</b>	<b>00800 161 2205 7799</b>
<b>IS</b>	<b>800 8996</b>
<b>LT</b>	<b>8800 30728</b>
<b>RO</b>	<b>0800 895 084</b>
<b>SK</b>	<b>0800 606 287</b>
<b>LI</b>	<b>+31 20 796 5692</b>
<b>MT</b>	<b>+31 20 796 5693</b>

## TABLE OF CONTENTS

Symbols used.....	2
Intended use .....	3
Test principle.....	3
Reagents.....	4
<i>Description, preparation for use, and recommended storage conditions</i> .....	4
Materials required but not provided.....	5
Safety and environment .....	6
Specimens .....	7
Remarks and precautions .....	7
Preparation and manipulation procedures .....	7
<i>Strip handling</i> .....	7
Manual test procedure.....	7
<i>Directions for incubation</i> .....	7
<i>Directions for changing liquid in the troughs</i> .....	8
<i>Hybridization</i> .....	8
<i>Stringent wash</i> .....	9
<i>Color development</i> .....	9
Automated test procedure.....	10
Results .....	10
<i>Reading</i> .....	10
<i>Interpretation of the results</i> .....	10
Quality control .....	11
Interpretation software: LiRAS for LiPA HPV .....	11
Limitations of the procedure.....	12
Test performance .....	12
<i>Amplification success rate</i> .....	12
<i>Clinical specificity</i> .....	12
<i>LiRAS for LiPA HPV software</i> .....	13
<i>Analytical sensitivity</i> .....	13
<i>Genotype inclusivity</i> .....	13
<i>Precision</i> .....	13
Recommendations on laboratory design and procedures.....	14
Trademarks .....	15
Licenses .....	15
Interpretation Chart .....	16

### Symbols used



Manufacturer



In Vitro Diagnostic Medical Device



Batch code



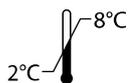
Catalogue number



Use By



Consult Instructions for Use



Temperature limitation



Contains sufficient for &lt;n&gt; tests

CONJ 100x

Conjugate 100x

CONJ DIL

Conjugate Diluent

DENAT SOLN

Denaturation Solution

HYBRIDIZ SOLN

Hybridization Solution

RINSE SOLN 5x

Rinse Solution 5x

STRIN WASH SOLN

Stringent Wash Solution

STRIPS

Strips

SUBS BCIP/NBT 100x

Substrate BCIP/NBT 100x

SUBS BUF

Substrate Buffer

### Intended use

The INNO-LiPA HPV Genotyping *Extra* is a line probe assay, for *in vitro* diagnostic use, designed for the identification of 28 different genotypes of the human papillomavirus (HPV) by detection of specific sequences in the L1 region of the HPV genome.

### Test principle

The INNO-LiPA HPV Genotyping *Extra* is based on the principle of reverse hybridization. Part of the L1 region of the human papillomavirus (HPV) genome is amplified using SPF10 primers, and the resulting biotinylated amplicons are then denatured and hybridized with specific oligonucleotide probes. An additional primer pair for the amplification of the human HLA-DPB1 gene is added to monitor sample quality and extraction. All probes are immobilized as parallel lines on membrane strips. After hybridization and stringent washing, streptavidin-conjugated alkaline phosphatase is added, which binds to any biotinylated hybrid previously formed.

Incubation with BCIP/NBT chromogen yields a purple precipitate and the results can be interpreted visually or by using the LiRAS<sup>®</sup> for LiPA HPV software.

An amplification kit (INNO-LiPA HPV Genotyping *Extra* Amp) is available for standardized preparation of biotinylated amplified material. This amplification kit is based on the polymerase chain reaction (PCR) using SPF10 primers.

The application of SPF10 in the INNO-LiPA HPV Genotyping *Extra* is protected by Innogenetics' EP patent 1012348B, US patent 6,482,588B and foreign equivalents.

Amplification products are subsequently hybridized using a single typing strip on which 28 sequence-specific DNA probe lines and 4 control lines are fixed (see Figure 1).

INNO-LiPA HPV Genotyping *Extra* assay: steps involved

Step 1 Amplification of the extracted DNA.

Step 2 Hybridization of the amplified product on the strip, followed by stringent wash.

Step 3 Addition of conjugate and substrate, resulting in color development.

Step 4 Visual interpretation of the signal pattern or use of the LiRAS for LiPA HPV Software.

## Reagents

### ***Description, preparation for use, and recommended storage conditions***

- If kept at 2 - 8°C, opened or unopened, and stored in the original vials, the reagents are stable until the expiry date of the kit. **Do not use the reagents beyond the expiry date. Do not freeze any of the reagents.**
- The reagents should be stored isolated from any source of contaminating DNA, especially amplified products.
- All reagents and the tube containing the strips should be brought to room temperature (20 - 25°C) approximately 60 minutes before use and should be returned to the refrigerator immediately after use.
- Alterations in physical appearance of the kit components may indicate instability or deterioration.
- To minimize the possibility that strips curl before use, it is recommended to store the tube horizontally.

Reagents supplied:

<b><u>Component</u></b>	<b><u>Quantity</u></b>	<b><u>Ref.</u></b>	<b><u>Description</u></b>
Strips	1x 20	59730	Containing 20 INNO-LiPA HPV Genotyping <i>Extra</i> strips marked with a red marker line.
Denaturation Solution	1x 1 ml	56718	Alkaline solution containing EDTA. This vial should be closed immediately after use; prolonged exposure of this solution to air leads to a rapid deterioration of the denaturing strength.
Hybridization Solution	1x 80 ml	57420	SSC buffer containing sodium lauryl sulfate (SLS), to be prewarmed to a temperature of at least 37°C and not exceeding 49°C.
Stringent Wash Solution	1x 200 ml	57421	SSC buffer containing SLS, to be prewarmed to a temperature of at least 37°C and not exceeding 49°C.
Conjugate 100x	1x 0.8 ml	56952	Streptavidin labeled with alkaline phosphatase in Tris buffer containing protein stabilizers and 0.01% MIT/0.098% CAA as preservative. To be diluted 1/100 in Conjugate Diluent: prepare 2 ml Conjugate working solution for each test trough + 2 ml in excess for manual testing. The Conjugate working solution is stable for 8 hours at room temperature (20 -25°C) if stored in the dark.
Conjugate Diluent	1x 80 ml	56951	Phosphate buffer containing NaCl, Triton <sup>®</sup> , protein stabilizers, and 0.01% MIT/0.1% CAA as preservative.

<b><u>Component</u></b>	<b><u>Quantity</u></b>	<b><u>Ref.</u></b>	<b><u>Description</u></b>
Substrate BCIP/NBT 100x	1x 0.8 ml	56954	BCIP and NBT in DMF. To be diluted 1/100 in Substrate Buffer before use: prepare 2 ml Substrate working solution for each test trough + 2 ml in excess for manual testing. The Substrate working solution is stable for 8 hours at room temperature (20 - 25°C) if stored in the dark.
Substrate Buffer	1 x 180 ml	56953	Tris buffer containing NaCl, MgCl <sub>2</sub> , and 0.01% MIT/0.1% CAA as preservative.
Rinse Solution 5x	1 x 80 ml	56721	Phosphate buffer containing NaCl, Triton <sup>®</sup> , and 0.05% MIT/0.5% CAA as preservative. To be diluted 1/5 in distilled or deionized water before use: prepare 8 ml Rinse working solution for each test trough + 10 ml in excess for manual testing. The Rinse working solution is stable for 2 weeks at 2 - 8°C.
Incubation trays	3	-	
Reading card	1	-	For identification of the positive probes.
Data reporting sheet	1	-	For storage of developed strips.

### **Materials required but not provided**

- INNO-LiPA HPV Genotyping *Extra* Amp.
- Distilled or deionized water.
- Disposable gloves.
- Disposable DNA/Dnase-free pipette tips (aerosol resistant).
- Forceps for strip handling.
- Graduated cylinders (10, 25, 50, and 100 ml).
- Adjustable pipettes to deliver 1 - 20 µl, 20 - 200 µl, and 200 - 1000 µl.
- Vortex mixer or equivalent.
- Microcentrifuge.

### **Materials required for the manual procedure only:**

- Water bath with shaking platform (80 rpm; with inclined lid; temperature adjustable to 49°C ± 0.5°C).
- Aspiration apparatus.
- Calibrated thermometer.
- Orbital, reciprocal, or rocking platform shaker.

### **Recommendations**

#### *For an orbital shaker:*

- The diameter of the circular motion should be equal to or greater than 13 mm.
- Recommended speed for a 13 mm circular motion is 160 rpm.

#### *For a reciprocal shaker:*

- Recommended speed for the to-and-fro motion is 80 movements per minute.

#### *For a rocking platform shaker:*

- The shaking angle should not exceed 13° to avoid spilling of liquid.

- Recommended speed is 50 rpm.
- Dispensing multipipette (Eppendorf, optional).
- Timer, 2 hours ( $\pm$  1 minute).

## Safety and environment

Please refer to the **Material Safety Data Sheet (MSDS)** and product labelling for information on potentially hazardous components. The most recent MSDS version is available on the Web site: [www.innogenetics.com](http://www.innogenetics.com).



**Toxic! (T)** R61-20/21-36, S53-9-20-23-36/37/39-45-60  
**Contains N,N Dimethylformamide:** SUBS BCIP/NBT 100x



**Irritant! (Xi)** R43, S23-24-37-60  
**Contains 2-Chloroacetamide:** RINSE SOLN 5x, SUBS BUF, CONJ DIL, HYBRIDIZ SOLN, STRIN WASH SOLN



**Corrosive! (C)** R34, S20-23-26-36/37/39-45-60  
**Contains sodium hydroxide:** DENAT SOLN

- R20/21 Harmful by inhalation and in contact with skin.  
R34 Causes burns.  
R36 Irritating to eyes.  
R43 May cause sensitization by skin contact.  
R61 May cause harm to the unborn child.  
S9 Keep container in a well-ventilated place.  
S20 When using do not eat or drink.  
S23 Do not breathe vapour/spray.  
S24 Avoid contact with skin.  
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.  
S36/37/39 Wear suitable protective clothing, gloves, and eye/face protection.  
S37 Wear suitable gloves.  
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).  
S53 Avoid exposure — obtain special instructions before use.  
S60 This material and its container must be disposed of as hazardous waste.
- Specimens should always be handled as potentially infectious. Therefore, all blood components and biological materials should be considered as being potentially infectious and should be handled as such. Only adequately trained personnel should be permitted to perform the test procedure.
  - All blood components and biological materials should be disposed of in accordance with one of the following established safety procedures:
    - Autoclave for at least 15 minutes at 121°C.
    - Incinerate disposable material.
    - Mix liquid waste with sodium hypochlorite so that the final concentration is  $\pm$  1% sodium hypochlorite. Allow to stand overnight before disposal.  
Caution: Neutralize liquid waste that contains acid before adding sodium hypochlorite.

- Use of personal protective equipment is necessary: gloves and safety spectacles when manipulating dangerous or infectious agents.
- Waste should be handled according to the institution's waste disposal guidelines. Also observe federal, state, and local environmental regulations.

### **Specimens**

Since the INNO-LiPA HPV Genotyping *Extra* test utilizes biotinylated amplified DNA material as specimen, an amplification kit, INNO-LiPA HPV Genotyping *Extra* Amp, is available as an accompanying tool.

### **Remarks and precautions**

- Do not mix reagents from different kits unless the components have identical lot numbers.
- Do not reuse disposable lab material.
- All vessels used to prepare conjugate and substrate solutions should be cleaned thoroughly and rinsed with distilled water.
- Avoid microbial contamination of reagents.
- Use a new DNA/DNase-free pipette tip for each aliquoted specimen. Sterile-packed, aerosol-resistant, disposable pipette tips are recommended.

### **Preparation and manipulation procedures**

#### ***Strip handling***

- The strips are designed to be used only once!
- Do not touch the strips with bare hands; use clean forceps.
- Use a **pencil** for identification of the test strips. Do not use ballpoints, etc. Write the ID above the marker line on the strips.
- Place the test strips in the troughs with their coated membrane side up (this side is marked).
- Test strips should always remain in the same trough throughout the different incubation steps.
- Unused or developed strips should be kept away from intense light and heat.
- Allow the developed strips to dry completely before interpretation, covering, and storing.
- Developed dry strips should be stored preferably in the dark at room temperature (20 - 25°C).
- Do not reuse the troughs.

### **Manual test procedure**

#### ***Directions for incubation***

- The hybridization and stringent wash incubations should be performed at **exactly 49°C** and are the most critical steps to avoid false-positive (temperature too low) or false-negative/very weak signals (temperature too high).  
A shaking water bath with **inclined** lid allows a good control of temperature variations. Strict temperature control (within 0.5°C from the set point of 49°C) with a calibrated thermometer is necessary.
- Always **close** the lid of the water bath during incubations in order to avoid false-positive signals.
- **Do not use a hot air shaker for the hybridization and stringent wash.**

- The amplitude of the motion generated by both the shaking water bath (hybridization and stringent wash procedure) and the orbital, reciprocal shaker or rocking platform (color development procedure) is critical in achieving maximum sensitivity and homogeneous staining. The amplitude should be as high as possible, so that both the liquid and the test strips move back and forth in the trough. However, spillage of liquid over the edges of the troughs must be avoided.
- For the hybridization and the stringent wash, the troughs should be placed on the shaking platform of the water bath. Adjust the water level to between 1/3 and 1/2 of the height of the trough. Make sure that the troughs do not float on the water. The water should be in direct contact with the troughs.
- Incubation steps for the color development should be performed at a temperature between 20 - 25°C.  
If the temperature is below 20°C, weaker results may be obtained. If the temperature is above 25°C, high background and/or false-positive signals may be obtained.
- The specified incubation times should be strictly respected in order to ensure correct performance of the assay.
- Do not cover the tray. During hybridization and stringent wash incubations, the troughs can be left uncovered in the water bath. Covering the troughs with microplate sealers may result in cross-contamination.

### ***Directions for changing liquid in the troughs***

- Aspirate the liquid from the trough with a pipette, preferably attached to a vacuum aspirator. Hold the tray at an angle to allow all liquid to flow to one end of the trough.
- Add 2 ml of the appropriate solution to each trough and follow the protocol.  
Note: A dispensing Multipette® (Eppendorf) is useful for this purpose.
- Repeat this step as many times as indicated in the test protocol.

#### **Note:**

- Do not allow the strips to dry between the washing steps.
- Make sure the surface of the strips is not damaged when aspirating. Preferably aspirate the liquid from the top of the strip above the marker line.
- Make sure the whole strip is thoroughly washed by complete submersion in the solution.
- Alter the speed of the shaker when necessary.

### ***Hybridization***

Note: Wear disposable gloves and use forceps.

1. Heat a shaking water bath to **exactly 49°C**. Check the temperature using a calibrated thermometer and adjust if necessary. Prewarm the Hybridization Solution and Stringent Wash solution to at least 37°C but do not exceed 49°C. Mix before use.
2. Using forceps, remove the required number of the test strips from the tube (1 strip per sample) and put an identification number above the marker line on the strip using a pencil.
3. Take the required number of test troughs (1 trough per strip) and place them in the tray.
4. Pipette 10 µl Denaturation Solution into the upper corner of each trough.  
Note: Close the vial immediately after use.

5. Add 10  $\mu$ l amplified biotinylated product to the Denaturation Solution and carefully mix by pipetting up and down several times. Always use DNA/DNase-free aerosol-resistant pipette tips. Allow denaturation to proceed for 5 minutes at room temperature (20 - 25°C).
6. **Shake the prewarmed Hybridization Solution** and **gently** add 2 ml to the denatured amplified product in each trough. Take care not to contaminate neighboring troughs during pipetting.
7. Immediately place the strip into the trough. The strips should be completely submerged in the solution.
8. Place the tray in the 49°C shaking water bath (approximately 80 rpm; see Directions for incubation), close the lid, and incubate for 60 minutes.  
Note: Avoid splashing water from the water bath into the trough. Adjust the water level to between 1/3 and 1/2 of the height of the trough.

### ***Stringent wash***

After hybridization, remove the tray from the water bath.

1. Hold the tray at a low angle and aspirate the liquid from the trough with a pipette, preferably attached to a vacuum aspirator. Add 2 ml prewarmed Stringent Wash solution to each trough and rinse by shaking the tray for 10 - 20 seconds at room temperature. Aspirate the solution from each trough.
2. Repeat this washing step once (see also Directions for changing liquid in the troughs).
3. Finally, aspirate the solution and incubate each strip in 2 ml prewarmed Stringent Wash solution in the shaking water bath at 49°C for 30 minutes. Close the lid of the water bath.  
Note: Prepare Rinse Solution and Conjugate during stringent wash incubation (see Reagents).

### ***Color development***

All subsequent incubations are carried out at **20 - 25°C on a shaker**.

1. Wash each strip twice for 1 minute using 2 ml diluted Rinse Solution (see Directions for changing liquid in the troughs). Aspirate.
2. Add 2 ml of Conjugate solution to each trough and incubate for 30 minutes while shaking. Aspirate.  
Note: Prepare Substrate solution about 10 minutes prior to the end of the conjugate incubation (see Reagents).
3. Wash each strip twice for 1 minute using 2 ml diluted Rinse Solution and wash once more using 2 ml Substrate Buffer. Aspirate.
4. Add 2 ml of Substrate solution to each trough and incubate for 30 minutes while shaking. Aspirate.
5. Stop the color development by washing the strips twice in 2 ml distilled water while shaking for at least 3 minutes.
6. Using forceps, remove the strips from the troughs and place them on absorbent paper. Let the strips dry completely and fix them to the data reporting sheet. The uppermost line is the marker line. The conjugate control line aids correct alignment of the strips on the data reporting sheet.

## Automated test procedure

Automated test procedure is available. Contact Innogenetics or your local distributor for further details.

## Results

### Reading

Figure 1 illustrates the position of the different oligonucleotide probes on the INNO-LiPA HPV Genotyping *Extra* strip. A line is considered positive when a clear purple/brown band appears at the end of the test procedure.



Figure 1: Location of the specific probes on the INNO-LiPA HPV Genotyping *Extra* strip. A marker line is present at the top of the strip for orientation.

### Interpretation of the results

The strips should only be read when they are completely dry.

All visible lines should be scored by using the INNO-LiPA HPV Genotyping *Extra* Reading Card.

The line patterns should be compared to the INNO-LiPA HPV Genotyping *Extra* Interpretation chart supplied with the kit. This chart marks the positive lines (rows) for the different HPV types (columns) with an "X".

All HPV types for which the type-specific line pattern (columns of the Interpretation chart) is a subset of the full line pattern observed on the strip must be scored as present or possibly present in the sample. An HPV type is possibly present in the sample if ALL probe lines that form its specific hybridization pattern are already part of a single or multiple specific hybridization patterns of other HPV types. This is the case for HPV18-(HPV39); HPV 31-(HPV 52)-(HPV 54); HPV 33-( HPV 52)-( HPV 54); HPV 40-( HPV 52); HPV 53-( HPV 52); HPV 56-( HPV 74); HPV 58-( HPV 52); HPV 68-(HPV 39) and HPV 73-( HPV 39)-( HPV 68). Genotypes between brackets are possibly present as co-infection.

Samples for which the obtained line pattern cannot be assigned to any genotype pattern or which have no type-specific lines (lines 1 - 28), but have at least one HPV control line positive, must be scored as HPV positive, but are untypeable (HPVX).

A sample for which only part of the obtained line pattern can be assigned to one or more particular genotypes contains HPV X (an untypeable HPV type) as well as these particular genotypes.

A reactivity on all probe lines should be considered as not interpretable. The whole procedure starting from the DNA extraction should be repeated for samples showing this pattern.

HPV Result	hDNA Result	Interpretation
-	-	<b>Invalid Result</b> A negative result on the hDNA control line indicates inadequate specimen collection, processing, or presence of inhibitors in the DNA extract. In the latter case, testing of a 1:10 dilution of the DNA extract can improve amplification performance. If not successful, start procedure from a new aliquot of the specimen.
-	+	<b>HPV not detected</b> A negative result on both HPV control lines and type-specific lines indicates the absence of HPV DNA, but cannot preclude the presence of an HPV infection.
+	-	<b>HPV detected</b> A positive result on at least one of the type-specific lines or HPV control lines indicates the presence of HPV DNA. The presence of additional HPV genotypes cannot be completely ruled out.
+	+	<b>HPV detected</b> A positive result on at least one of the type-specific lines or HPV control lines indicates the presence of HPV DNA. The presence of additional HPV genotypes cannot be completely ruled out.

### Quality control

- The first line (immediately below the marker line) is the Conjugate Control line. This line controls for the addition of reactive Conjugate and Substrate solution during the detection procedure. It should always be positive and should have approximately the same intensity on each strip in the same test run.
- The second line is a human DNA control line. Primers amplifying a fragment of the human HLA-DPB1 gene are added to the HPV amplification kit to monitor the sample quality and extraction efficiency. This line should always be positive except when the amplification of human DNA is outcompeted by the presence of a high amount of HPV DNA in the sample.
- A sample is considered HPV positive if at least one of the type-specific lines or one of the HPV control lines is positive.
- Always include a positive run control: e.g., the positive amplification control included in the INNO-LiPA HPV Genotyping *Extra* Amp kit. The positive control contains HPV6 and HLA-DPB1 and should react on the following lines: Conj Control, hDNA control, HPV control 1, and line 1 (HPV6).
- Always include a negative run control that is processed simultaneously with the patient samples in the DNA extraction and PCR step. If a positive band (except for the hDNA control in case of an HPV negative clinical sample) is obtained on the LiPA for the negative control, the entire run should be discarded and the complete procedure should be repeated.

### Interpretation software: LiRAS for LiPA HPV

The LiRAS for LiPA HPV software is designed to assist with the interpretation of the INNO-LiPA HPV Genotyping *Extra* results. Please contact your local distributor to obtain the latest version.

Always compare the strip pattern and patient information shown in the report with the original strip to ensure that the strip was identified, aligned, and scanned correctly.

### **Limitations of the procedure**

- The INNO-LiPA HPV Genotyping *Extra* does not allow discrimination between HPV69 and HPV71.
- Mixed HPV genotype infections are common. The INNO-LiPA HPV Genotyping *Extra* Amp kit uses a set of primers that amplify all genotypes simultaneously. Due to PCR competition and the absence of particular genotypes on strip, it is possible that certain genotypes present in the co-infected sample are not detected.
- Suboptimal amplification with SPF10 consensus PCR has been demonstrated for genotype 59 resulting in a higher detection limit.

### **Test performance**

The test performance, using visual interpretation, of the INNO-LiPA HPV Genotyping *Extra* was evaluated using a total of 200 cervical cell suspensions collected in Surepath<sup>®</sup> (BD- Tripath) preservative fluid. A reference method was performed for these samples, which was either the LINEAR ARRAY HPV Genotyping (Roche), or this test in combination with consensus primer-based (MY09-MY11 primers) and type-specific PCRs. Using these reference methods, 97 specimens were classified HPV positive (of which 93 HPV positive with a genotype that can be detected with the LiPA assay) while 103 specimens were considered to be HPV negative.

### **Amplification success rate**

Positive LiPA test results, as indicated by at least one positive HPV control line or at least one positive type-specific line, were obtained for 91 of 93 HPV-positive specimens (97.8%, 95% CI [92.0%; 99.9%]). This result was obtained both after initial amplification and testing on LiPA and after repeat LiPA testing in duplicate starting from extraction.

### **Clinical specificity**

A negative HPV test result, as indicated by a positive hDNA control line and no other positive line, was obtained for 99 of the 103 HPV-negative samples after initial amplification and testing on LiPA (96.1%; 95% CI [90.1%, 98.8%]).

All 4 samples with positive LiPA results were re-amplified and retested on LiPA in duplicate starting from extraction. Only 2 of the 4 samples were positive resulting in a clinical specificity of 98.1% (101/103; 95% CI [92.8%, 99.9%]) after repeat testing. These 2 samples were identified as HPV74, a genotype which cannot be detected by the LINEAR ARRAY HPV Genotyping. Both samples were also found negative by consensus PCR and type-specific PCR for HPV74.

### **High-risk genotype concordance after discrepancy testing**

Out of all 200 samples tested, 91 samples gave a positive initial LiPA result and were found to be HPV positive by either the LINEAR ARRAY HPV Genotyping test or PCR method. The type-specific PCR method was performed in case of discrepancy between the LiPA result and the LINEAR ARRAY HPV Genotyping result for a (probable) high-risk genotype (15 high risk genotypes and 3 probable high risk genotypes). The final reference high-risk genotype result of the sample was based on the consensus result of 3 different assays.

The high-risk genotype concordance was calculated by comparing the initial INNO-LiPA HPV Genotyping *Extra* result (or the consensus result of 3 LiPA results, if re-testing was performed) and the reference genotype result.

For this study, results were termed concordant if identical high-risk genotypes were obtained by both assays regardless of the concordance for low risk genotypes.

**High-risk genotype concordance after discrepancy testing** was 92.3% (84/91; 95% CI [84.7%, 96.5%]). Six out of the 7 discordant samples were found to contain multiple HPV genotypes and have at least one high-risk genotype in common using both genotyping methods. HPV 59 was not detected on 2 occasions in 2 partially concordant samples.

### **LiRAS for LiPA HPV software**

All 200 samples were interpreted both visually and using the LiRAS for LiPA HPV v2.0 software.

The initial agreement between results obtained from visual LiPA interpretation and the LINEAR ARRAY HPV Genotyping test result was not significantly different from the initial agreement between the results obtained from the software LiPA interpretation and the LINEAR ARRAY HPV Genotyping test result ( $p=1$ ).

### **Analytical sensitivity**

The limit of detection was determined for HPV genotypes 16, 18, 31, 45, and 52, representing five of the most prevalent genotypes. Plasmid DNA (in the presence of background human DNA), was diluted (1/3) ranging from 5000 copies/PCR reaction to 2 copies/PCR reaction. Each dilution was tested 8 times. Limit of detection is defined as a 95% probit value (point estimate) and ranged from 20 to 70 copies/PCR reaction depending on the genotype tested. The reactivity of the different probelines present in the genotype-specific pattern for HPV 18 and HPV 31 showed differences below 62 copies/PCR and resulted in an incorrect interpretation (HPV 39 and 52 respectively). This was not observed during the performance evaluation using clinical samples.

### **Genotype inclusivity**

The INNO-LiPA HPV Genotyping *Extra* kit identifies 28 different genotypes. This was evaluated by testing a panel of plasmid samples (28 different genotypes) containing approximately 1000 copies or 10000 copies per PCR reaction in the presence of background human DNA, in duplicate. For all genotypes except HPV 59, both replicates tested were positive at 1000 copies/PCR reaction. For HPV 59, both replicates were positive at 10 000 copies/PCR reaction.

### **Precision**

The precision of the INNO-LiPA HPV Genotyping *Extra* was analyzed with 8 DNA extracts obtained from clinical samples, tested in 14 replicates: on three lots of the INNO-LiPA HPV Genotyping Amp *Extra* and the INNO-LiPA HPV Genotyping *Extra*, at one site, in different runs, on two different days, by two different operators and on three different *Auto*-LiPA instruments. The 8 samples included 4 single-genotype samples and 4 samples with a mixed HPV infection.

A 111/112 (99.1%; 95% CI [94.6%, 99.9%]) inter- and intra-assay, inter-person, inter-instrument and inter-lot concordance was achieved. For one replicate of one sample a

possible genotype (HPV52) was not detected, but the predominant genotypes were detected.

### **Recommendations on laboratory design and procedures**

The following sequence of operations is recommended:

1. Preparation and aliquoting of PCR mixes.
2. Preparation of samples (DNA isolation).
3. Polymerase chain reaction.
4. Analysis of the biotinylated PCR products by reverse hybridization.

Personnel involved in steps 3 and 4 should not subsequently participate in work for steps 1 and 2 on the same day.

Similarly, after being involved in step 2, do not subsequently participate in work for step 1 on the same day.

To prevent contamination (e.g., with amplimers) of specimens and to avoid false-positive results, the procedure should be performed in three physically separated rooms, each with its own set of supplies and pipettes. One room is necessary for reagent preparation, another for sample preparation, and a third room for amplification and amplimer detection.

All equipment should be kept in the room where it is used and not be transferred between rooms.

Aerosol-resistant pipette tips should be used to prevent cross-contamination between specimens. For the same reason, wear disposable examination gloves and change them frequently.

#### ***Room 1 - storage and preparation of reagents***

This room and its equipment must be kept **free of DNA**. This room is only to be used for preparing PCR reagents. The Control PCR should not be brought into Room 1. The personnel involved should wear a clean laboratory coat, which must not be worn outside this room. Wear disposable gloves when handling reagents.

#### ***Room 2 - sample preparation***

This room and its equipment must be kept **free of amplimers**. The personnel involved in specimen processing should wear a clean laboratory coat, which must not be worn outside this room. During sample preparation, disposable examination gloves should be worn and changed frequently. Carefully uncap vials containing (processed) sample. Avoid opening more than one reaction vial containing sample at the same time.

To avoid contamination or to clean contaminated surfaces, it is recommended to clean pipettes and work surfaces with DNAZap™ (Ambion). Be aware that the use of DNAZap™ is only an additional precautionary measure, and the described recommendations on laboratory design and procedures should be followed as strictly as possible.

#### ***Room 3 - amplification and amplimer detection***

The personnel involved in amplification and amplimer detection should wear a clean laboratory coat, which must not be worn outside this room and must be changed daily. Wear disposable examination gloves when working with amplimers.

### **Trademarks**

- INNOGENETICS<sup>®</sup> and LiRAS<sup>®</sup> are registered trademarks of Innogenetics N.V.
- DNAZap<sup>™</sup> is a trademark of Ambion Inc., USA
- Triton<sup>®</sup> is a registered trademark of Union Carbide Corp., USA
- Multipette<sup>®</sup> is a registered trademark of Eppendorf AG, DE

### **Licenses**

The purchase of this product allows the purchaser to use it for the performance of diagnostic services for human *in vitro* diagnostics. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

### Interpretation Chart

Probe #	HPV genotypes																																				
	6 LR	11 LR	16 HR	18 HR	18 HR	26* pHR	31 HR	33 HR	35 HR	39 HR	40 LR	43 LR	44 LR	45 HR	45 HR	51 HR	52 HR	53* pHR	53* pHR	54 LR	56 HR	58 HR	58 HR	58 HR	58 HR	59 HR	66* pHR	68 HR	69/71**	70 LR	70 LR	73 HR	74**	82 HR			
1	X																																				
2		X																																			
3			X																																		
4				X																																	
5					X																																
6						X																															
7																														X							
8							X				X													X	X												
9							X	X												X																	
10							(X)***	X																													
11									X																												
12				X	X					X																		X						X			
13											X																										
14												X																									
15													X																								
16														X	X																						
17																X																					
18							X	X			X						X	X	X	X			X	X	X	X											
19																		X	X																		
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24																											X										
25															X													X				X	X				
26																														X	X						
27																																		X			
28																																					X

\* : 26, 53 and 66 are considered probable high-risk (pHR) genotypes according to Munoz et al. N Engl J Med 2003;348:518-27.

\*\* : 69, 71 and 74 are not classified as high-risk, probable high-risk or low risk genotypes according to Munoz et al. N Engl J Med 2003;348:518-27.

\*\*\* : probeline 10 may show weak reactivity when probelines 8 and 9 are positive; in this case the sample should be interpreted as genotype 31



## INNO-LiPA HPV Genotyping *Extra Amp*

研究用

IVD

Manufactured by:

INNOGENETICS N.V.  
Technologiepark 6  
9052 Gent  
Belgium  
☎+32-9 329 13 29  
BTW BE 0427.550.660  
RPR Gent



"Note changes highlighted"

Distributed by:

INNOGENETICS GmbH  
Hans-Böckler-Allee 20  
30173 Hannover  
Germany  
☎+49-511-8573931

INNOGENETICS s.a.r.l.  
Les Conquérants, Bât. Le Kilimandjaro  
8/10, avenue des Tropiques  
91940 Les Ulis  
France  
☎+33-1 69 07 48 34

INNOGENETICS S.r.l.  
Via Vaccareccia 39/A  
00040 Pomezia (Roma)  
Italy  
☎+39-06 965 28 700

INNOGENETICS Diagnóstica Iberia, S.L.U.  
Calle Tarragona 161, Planta 14  
08014 Barcelona  
Spain  
☎+34-93 270 53 00

INNOGENETICS N.V.  
Technologiepark 6  
9052 Gent  
Belgium  
☎+32-9 329 13 29

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## TABLE OF CONTENTS

Symbols used .....	3
Intended use .....	3
Test principle .....	3
Reagents .....	4
Description, preparation for use, and recommended storage conditions.....	4
Materials required but not provided .....	4
Safety and environment .....	4
Specimen collection and DNA extraction .....	5
Preparation of cervical cells .....	5
DNA isolation using proteinase K .....	5
Remarks and precautions .....	5
Test procedure .....	6
PCR mix preparation .....	6
PCR cycling .....	6
Results .....	7
Validation .....	7
Limitations of the procedure.....	7
Test performance .....	7
Recommendations on laboratory design and procedures .....	7
Trademarks.....	8
Licenses.....	8
References .....	8



## Symbols used



Manufacturer



*In Vitro* Diagnostic Medical Device



Batch code



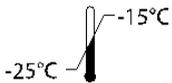
Catalogue number



Use By



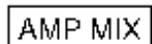
Consult Instructions for Use



Temperature limitation



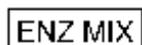
Amplification kit



Amplification Mix



Positive Control



Enzyme Mix

## Intended use

The INNO-LiPA HPV Genotyping *Extra Amp* kit, for *in vitro* diagnostic use, is designed to amplify part of the L1 region of the human papillomavirus (HPV) by using the polymerase chain reaction (PCR).

## Test principle

Amplification of a broad spectrum of HPV genotypes necessitates the use of consensus primers targeting a region in the HPV genome conserved between different genotypes. The most conserved region in the HPV genome is the L1 region, and several consensus PCR primer sets have been described in this region (Molijn et al. 2005). Examples are the GP5+/6+ (Jacobs et al. 1997), MY09/11 (Hildesheim et al. 1994) and PGMY (Gravitt et al. 2000) primer sets.

The SPF10 primer set used in the INNO-LiPA HPV Genotyping *Extra*, amplifies a 65-bp region in the L1 open reading frame (Kleter et al. 1998) and has the potential to amplify at least 54 HPV types (Safaeian et al. 2007).

The application of SPF10 is protected by Innogenetics' EP patent 1012348B, US patent 6,482,588B and foreign equivalents.

PCR amplification is performed in a reagent mixture containing an excess of deoxynucleoside 5'-triphosphates (dNTPs) including deoxyuridine triphosphate, biotinylated primers, thermostable DNA polymerase and uracil-N-glycosylase (UNG).

An incubation step prior to the amplification removes uracil bases from any contaminating amplification products present in the reaction mixture. The UNG enzyme is inactivated when the temperature is increased during the following denaturation step at 95°C. The sample mixture is heated in order to separate the two strands of the DNA helix (denaturation) and expose the target sequences to the primers. These primers are complementary to the regions flanking the target. In this way, two exact biotinylated copies of the template sequence are produced after one cycle of denaturation, annealing, and extension.

After 40 cycles, a multi-amplified biotinylated target sequence is obtained.



## Reagents

### **Description, preparation for use, and recommended storage conditions**

- If kept at -20°C, opened or unopened, and stored in the original vials, the reagents are stable until the expiry date of the kit. Do not use the reagents beyond the expiry date.
- The reagents should be stored isolated from any source of contaminating DNA, especially amplified products.
- To prevent contamination, store the Positive Control separately from amplification reagents and amplified material.
- Do not bring the enzyme mix to room temperature. Take it out of the freezer just before use and return to the freezer immediately after use. Spin down this vial before use.
- This reagent is viscous and therefore requires extra care in pipetting in order to deliver accurate volumes of reagent and to avoid wasting reagent. **Slowly release the plunger of the pipet so that the sample is correctly drawn into the pipet tip.**
- Bring the amplification mix to room temperature approximately 30 minutes before use and return to the freezer immediately after use.
- Alterations in physical appearance of the kit reagents may indicate instability or deterioration.

Reagents supplied:

<u>Component</u>	<u>Quantity</u>	<u>Ref.</u>	<u>Description</u>
AMP MIX	1 x 1.10 ml	59736	Contains biotinylated primers in buffer with dNTP/dUTP mix, MgCl <sub>2</sub> and 0.05% NaN <sub>3</sub> as preservative
ENZ MIX	1 x 0.061 ml	59734	Contains AmpliTaq Gold® 360 DNA polymerase and uracil-N-glycosylase
CONTROL +	1 x 0.05 ml	59732	PCR control contains HPV6 DNA and HLA-DPB1 DNA and 0.05% NaN <sub>3</sub> as preservative

### **Materials required but not provided**

- Materials for DNA extraction:
  - Proteinase K (Roche Diagnostics, Cat. No. 3115836, 25 mg)
  - Triton® X-100 (VWR, Cat. N° 1.08603.1000, 1l)
- Disposable gloves
- Disposable aerosol-resistant DNA/Dnase-free pipette tips
- DNA/DNase free microtubes
- DNAZap™ (Ambion, Cat. No. 9890)
- Microtube racks
- Microtube centrifuge
- Vortex mixer or equivalent
- Heating block
- DNA thermal cycler and equipment
- Pipettes adjustable to deliver 1 - 20 µl, 20 - 200 µl, and 200 - 1000 µl
- Mineral oil, silicone grease (if required)
- DNA/DNase-free deionized/distilled water (PCR grade)

### **Safety and environment**

- **Please refer to the Material Safety Data Sheet (MSDS) and product labeling for information on potentially hazardous components. The most recent MSDS version is available on the Web site: [www.innogenetics.com](http://www.innogenetics.com).**
- Specimens should always be handled as potentially infectious.
- Use of personal protective equipment is necessary:  
Wear gloves and safety spectacles when manipulating dangerous or infectious agents.
- Waste should be handled according to the institution's waste disposal guidelines.  
Also observe federal, state, and local environmental regulations.



- Amplification Mix and Positive Control contain sodium azide as preservative. To prevent the formation of very toxic gas, avoid contact of sodium azide with acids. To prevent the formation of explosive lead or copper azide in plumbing, thoroughly flush drains with water after disposal of solutions containing sodium azide.

### **Specimen collection and DNA extraction**

Collection of the sample, transport and subsequent DNA extraction are not part of the INNO-LiPA HPV Genotyping *Extra Amp* kit.

The INNO-LiPA HPV Genotyping *Extra Amp* kit has been validated using cervical cells collected in Surepath® medium followed by a proteinase K based DNA extraction procedure as described below. Standard protocols for cervical cell sampling in collection media (e.g. alcohol-containing solutions) combined with HPV DNA extraction using commercially available kits can be used but require in-house validation.

#### **Preparation of cervical cells**

- Loosen the cells from the brush by vortexing or rigorous mixing for 15 seconds.
- Transfer 1 ml of cervical cell suspension to a microtube, avoiding cross-contamination between samples.
- Spin the vials at approximately 13 000 rpm for 15 seconds.
- Discard the supernatant using a clean fine-tipped, disposable pipette for each reaction vial. Recap each vial.
- Add 1 ml of distilled water and vortex briefly to resuspend the cells.
- NOTE: resuspension of the cell pellet should be done in the same volume as the cervical cell suspension.

#### **DNA isolation using proteinase K**

##### *Preparation of Digestion Solution*

1. Proteinase K (400 µg/ml): 400 µg/ml in deionized water, aliquot and store at -20°C.
2. 6% Triton® X-100: 6% (v/v) in deionized water, aliquot and store at -20°C.
3. Digestion Solution: mix equal amounts of Proteinase K (400 µg/ml) and 6% Triton® X-100 immediately before use.

##### *Protocol*

- Pipette 50 µl Digestion Solution into microtubes and add 150 µl of the cell suspension.
- Vortex briefly.
- Incubate 1 hour at 56°C.
- Vortex briefly.
- Inactivate the Proteinase K for 10 minutes at 95°C.
- Vortex briefly.
- Use 10 µl for the PCR reaction and store the remainder at -20°C.

#### **Remarks and precautions**

- In order to avoid DNA contamination, a maximum physical separation between the pre- and post-amplification steps is recommended: separate rooms, separate pipettes and other lab material, separate lab coats and gloves (and their stock) are minimum precautions for prevention of contamination and part of good laboratory practice. The reagents should be isolated from any source of contaminating DNA, especially amplified DNA products. Also avoid microbial contamination of reagents.
- Avoid any return of materials from the post-amplification room to the pre-amplification room.
- All pipette tips and tubes used for the amplification process should be autoclaved. Aerosol-resistant pipette tips are recommended. Use a new DNA/DNase-free pipette tip for each aliquoted specimen.
- The reagents for amplification processes should be handled in a room free of DNA.
- After thawing, vortex AMP Mix and Positive Control, and spin down all reagents.



## Test procedure

### NOTE:

- This protocol was designed for optimal amplification in 0.2 ml PCR tubes in GeneAmp® PCR System 9700 thermal cyclers.
- This protocol can be used for most commercial types of thermal cyclers, but may require some modifications indicated by the manufacturer of the cycler.
- Prior to use, determine whether the protocol is compatible with the thermal cycler in use at your laboratory.
- Ensure the thermal cycler is calibrated prior to use.

### PCR mix preparation

It is very important to use the correct amount of each component. Too much or too little sample or reagents could result in aspecific amplification or even in no amplification at all.

IMPORTANT NOTE: Prepare the PCR mix on ice and avoid unnecessary delays in the setup of the run.

1. Determine the number of vials to be prepared (N) as:  
N = number of DNA samples + 1 (negative control; no DNA) + 1 (positive control) + 1  
Prepare a master mix for N samples in a DNA/Dnase-free 1.5 ml tube using aerosol-resistant pipette tips.

Composition of the master mix for 1 sample:

37.7 µl		AMP MIX
+ 2.3 µl		ENZ MIX

Spin down the vial ENZ MIX before use. This reagent is viscous and therefore requires extra care in pipetting in order to deliver accurate volumes of reagent and to avoid wasting reagent. **Slowly release the plunger of the pipet so the sample is correctly drawn into the pipet tip.**

2. Vortex briefly and spin down the reagents. Aliquot 40 µl of this master mix into (N-1) DNA/DNase-free amplification tubes. Cover the PCR mix with mineral oil if required.
3. Pipette 10 µl of the extracted material into the PCR mix. Add 10 µl of control DNA to the positive control tube. Add 10 µl of DNA/DNase-free distilled water to the negative control tube.
4. Place the samples into the preheated and calibrated thermal block (see manufacturer's instructions). Start the amplification program designed for the INNO-LiPA HPV Genotyping *Extra* amplification.

### PCR cycling

The correct temperature profile for the INNO-LiPA HPV Genotyping *Extra* amplification should be selected.

INNO-LiPA HPV Genotyping *Extra* PCR profile (cycler type: GeneAmp® PCR System 9700):

<u>step</u>	<u>temp</u>	<u>time</u>	
1 Decontaminate	37°C	10 min	Degradation of uracil containing DNA
2 Denature	94°C	9 min	Inactivation of UNG and activation of the <b>AmpliTaq Gold® 360</b> DNA polymerase
3 Denature	94°C	30 sec	<i>Repeat cycle</i> <i>steps 3 to 5</i> <i>40 times</i>
4 Anneal primers	52°C	45 sec	
5 Extend primers	72°C	45 sec	
6 Hold at 72°C			Duration < 2 hrs

Remove tubes from the thermal cycler, store the amplicon immediately at -20° ± 5°C or proceed immediately to the INNO-LiPA HPV Genotyping *Extra*.

NOTE: Do not store the amplified DNA products together with amplification reagents.



## Results

### **Validation**

- Include at least one positive and one negative control each time an amplification is performed. As with any new laboratory procedure, the inclusion of additional positive and negative controls should be considered until a high degree of confidence is reached in the ability to correctly perform the test procedure.
- If the inclusion of an additional positive control is desirable, use a known positive sample.

### **Limitations of the procedure**

- Use of this product should be limited only to personnel well trained in the techniques of amplification.
- Powder from disposable gloves and sodium hypochlorite have an inhibiting effect on amplification.
- Strongly hemolyzed samples may inhibit the PCR amplification and result in false-negative results.
- No experiments were performed to test the effect of possible interfering substances.
- Repeated freezing/thawing of the DNA samples might result in less efficient amplification.
- Specific amplification depends on good laboratory practice and careful performance of the procedures as specified under **Remarks and precautions** and under **Recommendations on laboratory design and procedures**.

### **Test performance**

See INNO-LiPA HPV Genotyping *Extra* insert.

### **Recommendations on laboratory design and procedures**

The following sequence of operations is recommended:

1. Preparation and aliquoting of PCR mixes.
2. Preparation of samples (DNA isolation).
3. Polymerase chain reaction.
4. Analysis of the biotinylated PCR products by reverse hybridization.

Personnel involved in steps 3 and 4 should not subsequently participate in work for steps 1 and 2 on the same day. Similarly, after being involved in step 2, do not subsequently participate in work for step 1 on the same day.

To prevent contamination (e.g., with amplimers) of specimens and to avoid false-positive results, the procedure should be performed in three physically separated rooms, each with its own set of supplies and pipettes. One room is necessary for reagent preparation, another for sample preparation, and a third room for amplification and amplimer detection. All equipment should be kept in the room where it is used and not be transferred between rooms.

Aerosol-resistant pipette tips should be used to prevent cross-contamination between specimens. For the same reason, wear disposable examination gloves and change them frequently.

#### **Room 1 - storage and preparation of reagents**

This room and its equipment must be kept **free of DNA**. This room is only to be used for preparing PCR reagents. The Positive Control should not be brought into Room 1. The personnel involved should wear a clean laboratory coat, which must not be worn outside this room. Wear disposable gloves when handling reagents.

#### **Room 2 - sample preparation**

This room and its equipment must be kept **free of amplimers**. The personnel involved in specimen processing should wear a clean laboratory coat, which must not be worn outside this room. During sample preparation, disposable examination gloves should be worn and changed frequently. Carefully uncap vials containing (processed) sample. Avoid opening more than one reaction vial containing sample at the same time.

To avoid contamination or to clean contaminated surfaces, it is recommended to clean pipettes and work surfaces with DNAZap™ (Ambion). Be aware that the use of DNAZap™ is only an additional



precautionary measure, and the described recommendations on laboratory design and procedures should be followed as strictly as possible.

### **Room 3 - amplification and amplimer detection**

The personnel involved in amplification and amplimer detection should wear a clean laboratory coat, which must not be worn outside this room and must be changed daily. Wear disposable examination gloves when working with amplimers.

### **Trademarks**

- INNOGENETICS® is a registered trademark of Innogenetics N.V.
- DNAZap™ is a trademark of Ambion Inc., USA
- PreservCyt® is a registered trademark of Hologic Inc., USA
- Surepath® is a trademark of BD Diagnostics, USA
- Triton® is a registered trademark of Pro Union Carbide Corp., USA
- AmpliTaq Gold® is a registered trademark of Roche Molecular Systems Inc, USA
- GeneAmp® is a registered trademark of Roche Diagnostics GmbH, and GeneAmp® is a registered trademark in the U.S. of Applied Biosystems
- QIAamp® is a registered trademark of the QIAGEN Group

### **Licenses**

The purchase of this product allows the purchaser to use it for amplification of nucleic acid sequences for human *in vitro* diagnostics in accordance with the patented method described in the package insert. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

This product is sold under licensing arrangements between Innogenetics N.V. and Invitrogen IP Holdings, Inc.

The purchase price of this product includes limited, nontransferable rights under European Patent Numbers 0401037; 0522884; and 0415755 and corresponding foreign patents, other than United States patents, owned by Invitrogen Corporation to use only this amount of the product to practice the claims in said patents solely for activities of the purchaser in detection of human papilloma virus (HPV) within the field of human diagnostics. No other rights are conveyed.

Further information on purchasing licenses under the above patents may be obtained by contacting the Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, CA 92008. Email: [outlicensing@invitrogen.com](mailto:outlicensing@invitrogen.com).

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