INNO-LiPA HPV Genotyping Extra Amp

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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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Ref.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP MIX</td>
<td>1 x 1.10 ml</td>
<td>59736</td>
<td>Contains biotinylated primers in buffer with dNTP/dUTP mix, MgCl₂ and 0.05% NaN₃ as preservative</td>
</tr>
<tr>
<td>ENZ MIX</td>
<td>1 x 0.061 ml</td>
<td>59734</td>
<td>Contains AmpliTaq Gold® polymerase and uracil-N-glycosylase</td>
</tr>
<tr>
<td>CONTROL +</td>
<td>1 x 0.05 ml</td>
<td>59732</td>
<td>PCR control contains HPV6 DNA and HLA-DPB1 DNA and 0.05% NaN₃ as preservative</td>
</tr>
</tbody>
</table>

**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, 1 l)
- 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.
- 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:
   \[ N = \text{number of DNA samples} + 1 \text{ (negative control; no DNA)} + 1 \text{ (positive control)} + 1 \]
   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):

<table>
<thead>
<tr>
<th>step</th>
<th>temp</th>
<th>time</th>
<th>notes</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>37°C</td>
<td>10 min</td>
<td>Degradation of uracil containing DNA</td>
</tr>
<tr>
<td>2</td>
<td>94°C</td>
<td>9 min</td>
<td>Inactivation of UNG and activation of the AmpliTaq Gold® DNA polymerase</td>
</tr>
<tr>
<td>3</td>
<td>94°C</td>
<td>30 sec</td>
<td>Repeat cycle steps 3 to 5</td>
</tr>
<tr>
<td>4</td>
<td>52°C</td>
<td>45 sec</td>
<td>40 times</td>
</tr>
<tr>
<td>5</td>
<td>72°C</td>
<td>45 sec</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>hold at 72°C</td>
<td>Duration &lt; 2 hrs</td>
<td></td>
</tr>
</tbody>
</table>

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.
**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.

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**References**