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digene® HPV Genotyping LQ Test, Amplification Kit Handbook

Σ 96

Version 1

IVD

For amplification of high-risk human papillomavirus (HPV) genotypes

CE

REF 613215

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Contents

Kit Contents

***digene* HPV Genotyping LQ Test, Amplification Kit**			**(96)**
Catalog no.			**613215**
Number of reactions			**96**
MM	Mastermix*		4 x 945 µl
POS	PCR Positive control†	CONTROL +	150 µl
NEG	PCR Negative control: water (PCR grade)		1000 µl
$MgCl_2$	$MgCl_2$ (25 mM)		1000 µl
	Handbook	HB	1

* Solution of GP5+ primer and biotinylated GP6+ primer, beta-globin primers, dNTP's, PCR buffer, HotStarTaq® *Plus* DNA Polymerase, and water.

†Solution of two plasmids containing an HPV18 and beta-globin sequence in T10E1 and carrier RNA.

Symbols

Contains sufficient for <N> tests

In vitro diagnostic medical device

European conformity

Catalog number

Manufacturer

Batch code

Material number

Important note

Temperature limitations

Use by

Consult instructions for use

Storage

The *digene* HPV Genotyping LQ Test, Amplification Kit should be stored immediately upon receipt at –20°C. Unopened vials are stable until the expiration date.

The *digene* HPV Genotyping LQ Test, Amplification Kit should be stored away from any source of contaminating DNA, especially amplified DNA products (see "Room 1," page 9). If the reagents are not fully used in one experiment, store the remainder at –20°C.

The PCR Positive Control should be stored separate from the rest of the kit at –20°C until the expiration date on the label.

Intended Use

The *digene* HPV Genotyping LQ Test, Amplification Kit is an accessory to the *digene* HPV Genotyping LQ Test, Detection Kit. It provides the Mastermix (MM), $MgCl_2$ solution, and the PCR Negative (NEG) and Positive (POS) Controls for the HPV PCR reactions.

Product Use Limitations

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the *digene* HPV Genotyping LQ Test, Amplification Kit is tested against predetermined specifications to ensure consistent product quality.

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the *digene* HPV Genotyping LQ Test,

Amplification Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/support/MSDS.aspx, where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

The *digene* HPV Genotyping LQ Test consists of 2 kits: the *digene* HPV Genotyping LQ Test, Amplification Kit and the *digene* HPV Genotyping LQ Test, Detection Kit. The *digene* HPV Genotyping LQ Test, Amplification Kit provides the reagents needed for the HPV PCR amplification. The *digene* HPV Genotyping LQ Test, Detection Kit enables easy and reliable identification of high-risk (HR) human papillomavirus (HPV) genotypes by hybridization, using xMAP technology on the LiquiChip® System.

Read the entire handbook before starting the protocol.

Principle

A highly conserved L1 sequence is amplified using the GP5+/6+ PCR primers. Amplification is performed using HotStarTaq *Plus* DNA Polymerase. The GP6+ primer is biotinylated, enabling detection and analysis of amplified sequences using the *digene* HPV Genotyping LQ Test, Detection Kit. Beta-globin primers allow co-amplification of human genomic DNA present in the clinical samples and function as an internal control for PCR inhibition and adequate sample and DNA purification.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Thin-walled PCR tubes, 0.2 ml
- Thermal cycler*
- Pipets *and disposable pipet tips with hydrophobic filters (1–20 µl, 20–200 µl, and 200–1000 µl)
- Optional: Multidispenser (for example, Finnpipette® Stepper from Thermo Electron, see www.thermo.com)* †

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Recommendations for 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. Amplification
4. Analysis of the biotinylated PCR products by reverse hybridization

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

To prevent contamination (for example, with amplification products) 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 needed for reagent preparation, another for sample preparation, and a third room for amplification and PCR product detection. All equipment should be kept in the room where it is used and should not be transferred between rooms. Filter tips should be used for pipetting to minimize cross-contamination between specimens. In addition, wear disposable gloves and change them frequently.

Room 1: This room should be used only for storage and preparation of PCR reagents. This room and its equipment must be kept free of amplification products. Laboratory personnel should wear a clean laboratory coat, which must not be worn outside this room. Disposable gloves should be worn at all times.

Room 2: This room is used for sample preparation and must be kept free of amplification products. Laboratory personnel should wear a clean laboratory coat, which must not be worn outside this room. During sample preparation, disposable gloves should be changed frequently. Carefully uncap vials containing processed sample to avoid cross-contamination. Avoid opening more than one reaction vial containing sample at the same time.

Room 3: This room is used for amplification and detection of PCR products. Laboratory personnel should wear a clean laboratory coat, which must not be worn outside this room and must be changed daily. When working with amplification products, disposable gloves should be worn.

The *digene* HC2 High-Risk HPV Test and the *digene* Genotyping LQ Test, Detection Kit can be performed in the same room. When doing so, perform the specimen processing, denaturation, and transfer to the hybridization plate for

the HC2 test prior to entering the HC2 and Genotyping LQ Detection testing laboratory (Room 3). This prevents the original specimen, which should be processed in Room 2, from exposure to amplification products used in Room 3.

Sample collection

The *digene* HPV Genotyping LQ Test, Amplification Kit has been validated for use with the following:

- *digene* Cervical Sampler (cat. no. 5122-1220)
- *digene* Female Swab Specimen Collection Kit (cat. no. 5123-1220)
- Specimens collected in Hologic ThinPrep® Pap Test PreservCyt® Solution
- STM (Denatured STM specimens stored according to the *digene* HC2 High-Risk HPV Test)

ⓘ Samples collected using other sampling devices or transported in other transport media have not been qualified for use with this assay.

Sample storage

Samples can be stored according to the information in Table 1, below. Incorrect sample storage could lead to false negative results with the assay.

Table 1. Sample storage conditions

Transport medium	Length of storage before HC2 testing	Temperature	Length of storage after HC2 testing	Temperature
STM	Up to 2 weeks	Room temperature (15–25°C)	Overnight	15–25°C
STM	Up to 3 weeks	2–8°C	Up to 3 months	–20°C

Table continued on next page.

Table 1. Continued

Transport medium	Length of storage before HC2 testing	Temperature	Length of storage after HC2 testing	Temperature
STM	From 3 weeks to 3 months	–20°C	Up to 3 months	–20°C
PreservCyt Solution	Up to 3 months	2–30°C	Overnight	2–8°C
PreservCyt Solution	Up to 3 months	2–30°C	Up to 3 months	–20°C

Sample DNA purification

ⓘ This process should be done before the protocol and in Room 2.

ⓘ Incorrect sample DNA purification procedures and an excess of freeze-thaw cycles could lead to false negative results with the assay.

Denatured STM and non-denatured PreservCyt samples can be used for the automated and manual methods.

For automated DNA purification, we validated the EZ1® DSP Virus Kit (cat.no. 62724). Samples that have been used in HC2 assays should be thawed completely, equilibrated to room temperature, and homogenized by brief vortexing before use. There should be a sample volume of 200 µl for all approved sample types. To purify DNA from denatured STM samples and non-denatured PreservCyt samples, add 3 µg carrier RNA to each sample, and select 90 µl as the elution volume.

For manual DNA purification, we validated the QIAamp® MinElute® Virus Spin Kit (50) (cat. no. 57704) with a starting volume of 200 µL for all approved sample types. Add 2.8 µg carrier RNA to each sample. Reference Table 1 *Volumes of Buffer AL and carrier RNA–Buffer AVE mix for the QIAamp MinElute Virus Spin Kit procedure* (page 17). Add ½ the volume of carrier RNA-AVE µl mixture to the volume of Buffer AL (ml) as specified in the table. Include recommended protocol steps 9 and 13 (see "Protocol: Purification of Viral Nucleic Acids from Plasma or Serum" in the *QIAamp MinElute Virus Spin Kit Handbook* (Third Edition, February 2007), Table 1, page 17 (Volume of Buffer AL and carrier RNA–Buf AVE mix required for the QIAamp MinElute Virus Spin Kit procedure) for more information). Elute DNA in 100 µl elution buffer. Both denatured STM and non-denatured PreservCyt samples can be used.

Protocol: PCR Amplification of HPV DNA

ⓘ Important points before starting

- Use the Mastermix (MM) and the $MgCl_2$ solution in Room 1 (an area separate from that used for sample preparation or PCR product analysis) and the PCR Positive (POS) and the PCR Negative (NEG) control in Room 2 (an area used for DNA preparation and separate from PCR product analysis).
- Commonly available DNA purification products should produce adequate DNA yield for this assay (see page 11).
- Read "Important Notes," starting on page 9.
- Set up all reaction mixtures in Room 1 (an area separate from that used for DNA preparation or PCR product analysis).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- HotStarTaq *Plus* DNA Polymerase requires an activation step of **5 min at 94°C** (see step 6 of this protocol).

Procedure

1. **In Room 1, thaw the Mastermix (MM) and 25 mM $MgCl_2$ at room temperature (15–25°C) or on ice.**
2. **Mix by gently vortexing.**

   ⓘ It is important to mix the solutions completely before use to avoid localized concentrations of salts.
3. **While still in Room 1, prepare a reaction mix according to Table 2, page 13.**

   ⓘ It is not necessary to keep reaction vessels on ice since HotStarTaq *Plus* DNA Polymerase is inactive at room temperature.

   ⓘ Store the mixes for a maximum of 2 hours.

   ⓘ The reaction mix contains all the components needed for PCR except the template DNA and the Positive/Negative (POS/NEG) Control.

   ⓘ Prepare a volume of reaction mix 10% greater than that required for the total number of PCR assays to be performed.
4. **Continuing in Room 1, mix the reaction mix thoroughly.**
5. **Dispense 40 µl of the reaction mix into each PCR tube.**

ⓘ Mix gently (for example, by pipetting the reaction mix up and down a few times).

Table 2. Composition of reaction mix for the amplification reaction

Component	Volume/reaction	Volume for 96 reactions*
Mastermix (MM)	33 µl	3485 µl
25 mM $MgCl_2$ solution	7 µl	739 µl
Total volume	**40 µl**	**4224 µl**
Template DNA/control	10 µl	

* Including 10% excess

6. **Go to Room 2 and add 10 µl template DNA to the individual tubes containing the reaction mix.**

   ⓘ The purified DNA should be completely thawed and homogenous prior to pipetting.

7. **Mix solution by gently pipetting up and down at least 2 times.**
8. **While still in Room 2, add 10 µl of each of the PCR Positive (POS) and the PCR Negative (NEG) Controls to individual tubes containing the reaction mix.**
9. **Mix by gently pipetting up and down at least 2 times.**

10. Go to Room 3 with sample PCR mix and program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 3, below.

ⓘ Each PCR program must start with an initial heat activation step at 94°C for 5 min. Do not exceed the 5 min activation time.

Table 3. Optimized cycling protocol

Initial activation step:*	**5 min**	**94°C**
3-step cycling		
Denaturation (with 2.8°C/s to 94°C):	20 s	94°C
Annealing (with 1.8°C/s to 38°C):	30 s	38°C
Extension (with 1.8°C/s to 71°C):	80 s	71°C
Number of cycles:	40	
Final extension:	4 min	71°C
Cool down:	10 s	10°C

* HotStarTaq *Plus* DNA Polymerase is activated by this heating step.

11. Place the PCR tubes in the thermal cycler and start the cycling program.

ⓘ If detection is not going to be performed on the same day, store tubes containing PCR products at –20°C. For the detection of the PCR product, use the detection kit as described in the digene *Genotyping LQ Test, Detection Kit Handbook*.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Low signals or sensitivity	
a) HotStarTaq *Plus* DNA Polymerase not activated	ⓘ Check whether PCR was started with an initial incubation step at 94°C for 5 min.
b) Pipetting error or missing reagent	ⓘ Repeat the PCR. Check the concentrations and storage conditions of reagents.
c) Problems with starting template	ⓘ Check the concentration, storage conditions, and quality of the starting template (see Appendix, page 16). If necessary, make serial dilutions of template DNA. Repeat the PCR using the new dilutions.
d) Problems with the thermal cycler	ⓘ Check the power to the thermal cycler and that the thermal cycler has been correctly programmed.

Appendix: DNA Starting Templates

Since PCR consists of multiple rounds of enzymatic reactions, it is more sensitive to impurities such as proteins, phenol/chloroform, salts, ethanol, EDTA, and other chemical solvents than single-step enzyme-catalyzed processes. QIAGEN offers a complete range of nucleic acid preparation systems, ensuring the highest-quality templates for PCR.

For more information about QIAGEN kits for DNA purification, contact one of our Technical Service Departments (see back cover or visit www.qiagen.com).

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
digene HPV Genotyping LQ Test, Amplification Kit (96)	For 96 reactions: GP5+/GP6+ Primers, Reagents and Buffers	613215

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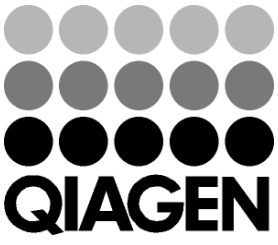

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