



April 2024

QIAscreen[®] HPV PCR Test Instructions for Use (Handbook)



For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

For the detection of human papillomavirus (HPV) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68.

For use with Rotor-Gene[®] Q 5plex platform instruments (MDx, 5plex and 5plex HRM)

REF 617006



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Intended Use

For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

The QIAscreen[®] HPV PCR Test is an in vitro real-time PCR-based assay for the qualitative detection of human papillomavirus (HPV) DNA of the following 15 (probably) high-risk HPV genotypes, i.e., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68.

The assay can be used to detect the presence of the above mentioned HPV types in genomic DNA specimens and can be used on the Rotor-Gene[®] Q 5plex platform instruments (i.e. MDx, 5plex and 5plex HRM).

Summary and Explanation

Human papillomaviruses (HPV) belong to the family of Papillomaviridae and are small double-stranded DNA viruses. The circular genome is approximately 7.9 kilo bases in size. More than 100 types of HPV have been identified, of which certain HPV types, known as high-risk HPV (hrHPV) like HPV 16 and 18, are associated with the induction of mucosal lesions that can progress to malignancy.

The viral genome contains early (E) and late (L) genes, which encode proteins necessary for early and late stages of the HPV life cycle, respectively. The E6 and E7 gene products of hrHPV types have carcinogenic properties and are necessary for malignant transformation of the host cell (1). Malignant progression is often associated with viral integration into the genome of the host cell (1). Integration results in interruption of the viral genome in a region that may extend from the E1 to the L1 open reading frame (1). This may have consequences for PCR-mediated amplification of viral DNA in these regions. As initiation and maintenance of the transformed phenotype depend on continuous expression of the viral oncoproteins (Horner, S.M., DeFilippis, R.A., Manuelidis, L., DiMaio, D. (2004) Repression of the human papillomavirus E6 gene initiates p53-dependent, telomerase-independent senescence and apoptosis in HeLa cervical carcinoma cells. *J. Virol.* 78, 4063., Butz, K., Ristriani, T., Hengstermann, A., Denk, C., Scheffner, M., Hoppe-Seyler, F. (2003) siRNA targeting of the viral E6 oncogene efficiently kills human papillomavirus-positive cancer cells. *Oncogene* 22 (38), 5938.), the viral E6/E7 region is invariably retained in integrated viral genomes in cervical cancers (1). The QIAscreen HPV PCR Test targets a conserved region within the E7 gene.

Principle of the Procedure

The QIAscreen HPV PCR Test is a multiplex, real-time PCR-based assay directed against the E7 gene of 15 (probably) hrHPV types that uses fluorescent probes for the detection of one or more accumulating PCR products. During each PCR cycle, the fluorescent signal increases in a logarithmic manner, resulting in an amplification curve. As soon as the amplification curve of the target surpasses its threshold, the sample is considered positive for that target. The multiplex format allows the simultaneous detection of four different fluorescent dyes per reaction, with each fluorescent dye representing different targets. The four different targets are: 1. HPV 16, 2. HPV 18, 3. the 13 other hrHPV types as a pool and 4. the human β -globin gene. The QIAscreen HPV PCR Test separately detects HPV 16, HPV 18, and the pool of 13 other hrHPV genotypes. The human β -globin gene is used as the sample control determining both the quality of the sample DNA and the presence of potential inhibitory substances.

Materials Provided

Kit contents

QIAscreen HPV PCR Test		72
Catalog no.		617006
Number of reactions		72
QIAscreen Master Mix (1 tube)	Transparent color	1080 µL
QIAscreen Positive Control (1 tube)	Transparent color	100 µL
QIAscreen Negative Control (1 tube)	Transparent color	100 µL
<i>QIAscreen HPV PCR Test Instructions for Use</i>		1

Materials Required but Not Provided

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

Consumables, reagents, and instruments for sample preparation

- Hologic PreservCyt[®] Solution (for self-collection sample storage)
- Standard DNA extraction kits, such as QIAamp[®] DSP virus spin kit (QIAGEN, cat. no. 61704) and QIAasymp[®] DSP Virus/Pathogen Midi Kit (QIAGEN, cat. no. 937055) and NucleoMag 96 Tissue kit (Macherey-Nagel, cat. no. 744300)
- PBS for handling cervical specimens in PreservCyt collection medium
- AL buffer (QIAGEN, cat.no. 19075) for pretreatment of cervical specimens collected in SurePath and CellSolutions collection medium

Consumables for the Rotor-Gene Q MDx Instrument

- 0.1 mL Strip Tubes and Caps, for use with 72-well rotor (QIAGEN, cat. no. 981103 or cat. no. 981106)

Equipment

- Dedicated pipets¹ (adjustable) for PCR (1–10 µL; 10–100 µL)
 - Dedicated filter-plugged sterile DNase-free pipette-tips
 - Disposable gloves
 - Benchtop centrifuge*
 - Vortex mixer*

Equipment for extraction and real-time PCR

- QIAAsymphony SP Module (cat. no. 9001297) (for optional automation of the extraction)
- Rotor-Gene Q 5plex HRM System (cat. no. 9002033) or Rotor-Gene Q MDx 5plex HRM instrument (cat. no. 9002033) with Rotor-gene Q software version 2.3.1 or higher²
- QIAscreenrun template for Rotor-Gene Q. The template is named QIAscreen RGQ profile v1.0.ret.
- QIAscreenchannel analysis templates for the channels green (HPV 16), yellow (HPV Other), orange (β-globin), and red (HPV 18). The templates have the file extension“.qut”.

¹Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

²If applicable, Rotor-Gene Q 5plex HRM instrument with a production date of January 2010 or later. The production date can be obtained from the serial number on the back of the instrument. The serial number is in the format “mmyynnn”, where “mm” indicates the production month in digits, “yy” indicates the last two digits of the production year, and “nnn” indicates the unique instrument identifier.

Warnings and Precautions

Safety information

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

- QIAscreen HPV PCR Test positive and negative controls contain sodium azide as a preservative (0.01%). Sodium azide may react with lead and copper plumbing to form explosive metal azides. On disposal through the sink, flush drains with generous amounts of cold water to prevent azide build-up.

General precautions

Use of PCR tests requires good laboratory practices, including maintenance of equipment, that are dedicated to molecular biology and are compliant with applicable regulations and relevant standards.

Always pay attention to the following:

- Wear protective disposable powder-free gloves, a laboratory coat, and eye protection when handling specimens.
- Prevent microbial and nuclease (DNase) contamination of the specimen and the kit. DNase may cause degradation of the DNA template.
- Avoid DNA or PCR product carryover contamination, which could result in a false-positive signal.

- Always use DNase-free disposable pipet tips with aerosol barriers.
- Reagents of the QIAscreen HPV PCR Test are optimally diluted. Do not dilute reagents further as this may result in a loss of performance.
- All reagents supplied in the QIAscreen HPV PCR Test are intended to be used solely with the other reagents supplied in the same kit. Do not substitute any reagent from one kit with the same reagent from another QIAscreen HPV PCR Test, even from the same batch, as this may affect performance.
- Refer to the Rotor-Gene Q MDx instrument user manual for additional warnings, precautions, and procedures.
- Before the first run of the day, perform a warm-up run for Rotor-Gene Q MDx 5 plex HRM at 95°C for 10 minutes.
- Alteration of incubation times and temperatures may result in erroneous or discordant data.
- Do not use components of the kit that have passed their expiration date or that have been incorrectly stored.
- Minimize exposure of the components to light; reaction mixes may be altered due to exposure.
- Use extreme caution to prevent contamination of the mixes with the synthetic materials that are contained in the PCR reagents.
- Discard sample and assay waste according to your local safety procedures.

Reagent Storage and Handling

Shipping conditions

The QIAscreen HPV PCR Test is shipped on dry ice and must be frozen on arrival. If any component of the QIAscreen HPV PCR Test is not frozen on arrival, the outer packaging has been opened during transit, or the shipment does not contain a packing note, handbooks, or the reagents, please contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Storage conditions

The QIAscreen HPV PCR Test must be stored immediately at -30°C to -15°C upon receipt in a constant-temperature freezer and protected from light.

Stability

When stored under the specified storage conditions, the QIAscreen HPV PCR Test is stable until the stated expiration date on box label.

Once opened, reagents can be stored in their original packaging at -30°C to -15°C . Repeated thawing and freezing should be avoided. Do not exceed a maximum of 5 freeze-thaw cycles.

- Gently mix by inverting the tube 10 times and centrifuge all tubes before opening.
- Expiration dates for each reagent are indicated on the individual component labels. Under correct storage conditions, the product will maintain performance for the stability time as long as the same batches of components are used.
- Quality control procedures at QIAGEN employ functional kit release testing for each individual kit lot. Do not mix reagents from different kits, even if they are from the same lot.

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

Specimen Storage and Handling



All specimens must be treated as potentially infectious material.

Cervical specimens

Collect and transport cervical specimens for testing with the QIAscreen HPV PCR Test using one of the following specimen collection media: PreservCyt, CellSolutions[®], Pathtezt[®], or Surepath[®] collection medium. All cervical specimens require preceding genomic DNA extraction before testing with the QIAscreen HPV PCR Test. Optimal storage temperature of the clinical samples is 2–8°C upon arrival at the lab. Under these storage conditions, samples in PreservCyt collection medium are stable for 3 months and samples in Surepath collection medium are stable for 2 weeks prior to DNA extraction.

Cervical samples collected in PreservCyt can be stored up to 210 days after sample collection at 18–25°C, up to two and a half years at 2–8°C, and up to 2 years at <20°C. Cervical samples collected in Surepath can be stored up to 10 weeks after sample collection at 2–30°C, up to two and half years at 2–8°C, and up to 210 days at <20°C.

Self-collected vaginal brush specimens

Self-collected vaginal brush specimens can be collected and shipped dry or in saline (0.9% w/v NaCl) and, upon arrival in the laboratory, stored in PreservCyt. Self-collected cervico-vaginal lavage specimens are collected and shipped in saline (0.9% w/v NaCl) and, upon arrival in the laboratory, stored in PreservCyt. All self-collected vaginal specimens require preceding genomic DNA extraction before testing with the QIAscreen HPV PCR Test. Self-samples in PreservCyt can be stored up to 210 days after sample collection at 18–25°C, up to two and half years at 2–8°C, and up to 2 years at <20°C.

Genomic DNA samples

Once genomic DNA is extracted, it can be stored at 2–8°C for short-term storage (≤ 2 days) or at –30°C to –15°C for up to 12 months.

Sample Preparation

DNA extraction

Standard DNA extraction kits (e.g., column- and magnetic bead-based kits, such as QIAamp DSP Virus spin kit, QIAasympphony DSP Virus/Pathogen Midi Kit, and NucleoMag 96 Tissue kit, (Macherey-Nagel) are compatible with this assay. Operating details for the QIAasympphony DSP Virus/Pathogen Midi are indicated below.

Clinical specimens in PreservCyt or PathTezt collection medium

For cervical specimens (scrapes) suspended in PreservCyt PathTezt collection medium, the fraction of DNA to be used as input in the PCR represents 0.125% of the 20 mL PreservCyt or PathTezt cervical scrape sample. This corresponds with 25 μ L of the original sample types. Since at maximum only 5 μ L of extracted DNA can be used as input in the PCR, DNA extraction procedures should be executed such that 5 μ L DNA extract corresponds with 25 μ L cervical specimen (scrape) sample to ensure that the correct fraction of the cervical sample is used in the PCR. Equivalent collection media should be processed similarly.

Important: PreservCyt medium can interfere with the DNA extraction process. This can be overcome in two different ways.

1. Dilute the aliquot of the PreservCyt specimen in an equal volume of PBS or lysis buffer from the DNA extraction kit and mix, before starting the DNA extraction. Make sure the total sample volume is compatible with the DNA extraction kit. If the total volume becomes too high for the extraction kit, it is advised to use method 2, outlined below.
2. Centrifuge the PreservCyt sample ($\geq 3400 \times g$ for 10 min) and remove the supernatant. The pellet is resuspended in appropriate volume of PBS or lysis buffer that is compatible

with the DNA extraction kit (for the QIAamp DSP Virus spin kit: resuspend in 200 µL PBS and follow the manufacturer's instruction for DNA extraction, elute in 100 µL; for the Margery Nagel Nucleomag96 tissue kit: resuspend in 100 µL buffer T1 of that kit and follow the manufacturer's instructions, elute in 100 µL).

Equivalent media should be processed similarly.

Operating details QIASymphony DSP Virus/Pathogen Midi Kit

QSDSP protocol: 500 µL of cervical sample in PreservCyt is mixed with 500 µL PBS. An Integrated run performing the Complex800_V6_DSP protocol is started on the QIASymphony following the steps described in 'QIASymphony® SP/AS Consolidated Operating Guide – 12.3 Integrated run'. DNA is eluted in 60 µL and 5 µL is used for the QIAScreen HPV PCR Test. If you only use the Qiasymphony SP module an sample preparation run performing the Complex800_V6_DSP protocol is started on the QIASymphony following the steps described in '*QIASymphony DSP Virus/Pathogen Kit Instructions for Use (Handbook) – General purification protocol*'.

For cervical specimens (scrapes) suspended in SurePath or CellSolutions collection medium, the fraction of DNA to be used as input in the PCR represents 0.25% of the 10 mL SurePath or CellSolutions cervical scrape sample. This corresponds with 25 µL of the original sample. Since at maximum only 5 µL of extracted DNA can be used as input in the PCR, sample volume and DNA elution volume should be selected such that 5 µL DNA extract corresponds with 25 µL cervical specimen (scrape) sample to ensure that the correct fraction of the cervical sample is used in the PCR.

Important: Clinical specimens collected in SurePath and CellSolutions medium must be pretreated prior to use to overcome formaldehyde-induced crosslinking using the described protocol below.

Pretreatment clinical specimens collected in SurePath and CellSolutions medium:

3. Mix the SurePath or CellSolutions specimen with a 1:1 volume of AL buffer (QIAGEN) and mix thoroughly.
4. Incubate at 90°C for 20 minutes followed by equilibration to room temperature before proceeding with DNA extraction.

Equivalent media containing formaldehyde should be processed similarly.

For self-collected vaginal brush specimens suspended in Hologic PreservCyt Solution, DNA extraction procedures should be executed such that 5 µL DNA extract used as input in the PCR represents 0.5% of the vaginal sample volume. For example, if the vaginal self-sample will be suspended in 2 mL PreservCyt Solution, then 5 µL input DNA corresponds with 10 µL of the self-sample suspension.

For self-collected cervico-vaginal lavage specimens, the fraction of DNA to be used as input in the PCR represents 0.5% of the lavage self-sample volume. Thus, in the case of a total lavage volume of 3 mL, DNA extraction procedures should be executed such that 5 µL input DNA corresponds with 15 µL of the original lavage self-sample.

Protocol: QIAscreen HPV PCR Test in the Rotor-Gene Q 5plex instrument

Important points before starting

Take time to familiarize yourself with the Rotor-Gene Q 5plex instrument before starting the protocol. See the instrument user manual.

Before the first run of the day, perform a warm-up run for the Rotor-Gene Q 5plex at 95°C for 10 minutes.

A Rotor-Gene Q series software template is required to run the test. Make sure the template QIAscreen RGQ profile v1.0.ret is used.

To analyze the test for each of the four detection channels, a Rotor-Gene Q series software template is required. Ensure that the correct template is used for each channel, as indicated below:

- **“QIAscreen RGQ Green Channel analysis template.qut”** must be used for analysis of the signals in the Green channel (HPV 16).
- **“QIAscreen RGQ Orange Channel analysis template.qut”** must be used for analysis of the signals in the Orange channel (β -globin).
- **“QIAscreen RGQ Yellow Channel analysis template.qut”** must be used for analysis of the signals in the Yellow channel (HPV Other).
- **“QIAscreen RGQ Red Channel analysis template.qut”** must be used for analysis of the signals in the Red channel (HPV 18).

Sample processing on Rotor-Gene Q 5 plex instruments with 72-tube rotor

Up to 70 genomic DNA samples can be tested within the same experiment, besides a positive and a negative control. The schematic in Table 1 provides an example of the loading block or rotor setup for an experiment with the QIAscreen HPV PCR Test. Numbers denote positions in the loading block and indicate final rotor position.

Table 1. Plate and rotor setup for an experiment with the QIAscreen HPV PCR Test on Rotor-Gene Q 5plex instrument

Strip	Tube position	Sample name	Strip	Tube position	Sample name	Strip	Tube position	Sample name
1	1	Positive Control	7	25	Sample 23	13	49	Sample 47
	2	Negative Control		26	Sample 24		50	Sample 48
	3	Sample 1		27	Sample 25		51	Sample 49
	4	Sample 2		28	Sample 26		52	Sample 50
2	5	Sample 3	8	29	Sample 27	14	53	Sample 51
	6	Sample 4		30	Sample 28		54	Sample 52
	7	Sample 5		31	Sample 29		55	Sample 53
	8	Sample 6		32	Sample 30		56	Sample 54
3	9	Sample 7	9	33	Sample 31	15	57	Sample 55
	10	Sample 8		34	Sample 32		58	Sample 56
	11	Sample 9		35	Sample 33		59	Sample 57
	12	Sample 10		36	Sample 34		60	Sample 58
4	13	Sample 11	10	37	Sample 35	16	61	Sample 59
	14	Sample 12		38	Sample 36		62	Sample 60
	15	Sample 13		39	Sample 37		63	Sample 61
	16	Sample 14		40	Sample 38		64	Sample 62

Table 1. Plate and rotor setup for an experiment with the QIAscreen HPV PCR Test on Rotor-Gene Q 5plex instrument (continued)

Strip	Tube position	Sample name	Strip	Tube position	Sample name	Strip	Tube position	Sample name
5	17	Sample 15	11	41	Sample 39	17	65	Sample 63
	18	Sample 16		42	Sample 40		66	Sample 64
	19	Sample 17		43	Sample 41		67	Sample 65
	20	Sample 18		44	Sample 42		68	Sample 66
6	21	Sample 19	12	45	Sample 43	19	69	Sample 67
	22	Sample 20		46	Sample 44		70	Sample 68
	23	Sample 21		47	Sample 45		71	Sample 69
	24	Sample 22		48	Sample 46		72	Sample 70

Note: Fill all unused positions with empty tubes.

PCR on Rotor-Gene Q 5 plex instruments with 72-tube rotor

1. Set up the **QIAscreen HPV PCR Test**.

Note: To minimize the risk for PCR reaction contamination, we strongly recommend to use a PCR-cabinet with UV-irradiation capability.

Important: Dispensing of the QIAscreen Master Mix must be performed in an area separate from that in which the DNA extraction is performed.

- a. Clean the bench area, pipets, and tube rack prior to use with a DNA-degrading solution to prevent template or nuclease contamination.

Note: Change tips between each tube to avoid any nonspecific template or reaction mix contamination which may lead to false-positive results.

- b. Mix gently by inverting 10 times, then briefly centrifuge before use to collect the solution at the bottom of the tube.
- c. Dispense 15 μL of the QIAscreen Master Mix into the appropriate tubes of the tube-strips (at maximum, 72 tubes per Rotor-gene Q 5plex run). Reaction setup can be done at room temperature.
- d. Return the QIAscreen Master Mix to the freezer to avoid any material degradation. Transport tubes to separate area to dispense the QIAscreen Positive Control and sample DNA.
- e. Add 5 μL of the negative control to tube position 2, mix by pipetting up and down or by flicking the tube, and close the tube by pressing the cap on the tube.
- f. Add 5 μL of the QIAscreen Positive Control to tube position 1, mix by pipetting up and down or by flicking the tube, and close the tube.

Note: Change tips between each tube to avoid any nonspecific template or reaction mix contamination, which may lead to false-positive results.

- g. Add 5 μL of sample DNA to the appropriate tubes containing the QIAscreen Master Mix, mix by pipetting up and down or by flicking the tubes, and close the tubes by pressing the caps on the tubes.
- h. Once a set of 4 tubes have been filled, cap the tubes.

Note: The PCR tubes can be stored for 30 minutes between pipetting samples into the PCR tubes and start of the experiment in the machine at 2–8°C in the dark.

- 2. Prepare the Rotor-Gene Q 5 plex and start experiment as follows:

Important: Before the first run of the day, perform a warm-up run for the Rotor-Gene Q 5 plex at 95°C for 10 minutes.

- a. Place a 72-well rotor on the rotor holder.
- b. Fill the rotor with strip tubes according to the assigned positions, starting at position 1, as shown in Table 1, with empty capped strip tubes placed into all unused positions.
Note: Ensure that the first tube is inserted into position 1 and the strip tubes are placed in the correct orientation and positions as shown in Table 1.
- c. Attach the locking ring.
- d. Load the Rotor-Gene Q 5plex instrument with the rotor and locking ring, and close the instrument lid.
- e. Go to New Run window and click **Open a template in another folder**.
- f. Select the QIAscreen run template named **QIAscreen RGQ profile v1.0.ret**.
- g. Select Rotor type: **72-well rotor** and **Locking ring attached**, and click **Next**.
- h. At operator, enter initials, and click **Next**.
- i. In the following window, click **Next**.
- j. Click **Start run**.

To enter sample names, click **Edit samples** (this can also be done after the run is completed).

Table 2. Target and channel settings

Target	Detection Channel
β-globin	Orange
HPV 16	Green
HPV 18	Red
HPV Other*	Yellow

* HPV Other comprises the pool of 13 non-16/18 HPV types

3. Analyze the data.
 - a. Select the tubes to be used for analysis.
 - b. Check the fluorescence data for each reaction for each dye for typical amplification curves (logarithmic increase of fluorescence). If the baseline of a sample does not provide satisfactory results, it is advised to repeat the PCR of this sample.
 - c. Go to Analysis tool window, select **Cycling A. Green**, and click **Show**. Click **Import** under Imported Settings (right bottom of window) and select the file **QIAScreen RGQ Green Channel analysis template.qut**. Select **Cycling A. Green** and click **Hide**.
 - d. Select **Cycling A. Orange** and click **Show**. Click **Import** under Imported Settings and select the file **QIAScreen RGQ Orange Channel analysis template.qut**. Select **Cycling A. Orange** and click **Hide**.
 - e. Select **Cycling A. Red** and click **Show**. Click **Import** under Imported Settings and select the file **QIAScreen RGQ Red Channel analysis template.qut**. Select **Cycling A. Red** and click **Hide**.
 - f. Select **Cycling A. Yellow** and click **Show**. Click **Import** under Imported Settings and select the file **QIAScreen RGQ Yellow Channel analysis template.qut**.
 - g. Click **Save**.
 - h. **OPTIONAL**: For interpretation of the results, the data can be exported as a **.csv** file. Go to **File > Save as > Excel Analysis Sheet** and save the export file.
4. Unload the Rotor-Gene Q 5plex instrument and discard the strip tubes according to your local safety regulations.

Interpretation of Results

The run and sample validation criteria are indicated below under A and B, respectively. Appropriate measures are indicated in case one (or more) criteria are not met.

A. Validation criteria of QIAscreen HPV PCR Test controls

Targets in the QIAscreen Positive Control should give C_T values that are lower than 29 for β -globin, lower than 30 for HPV 16 and HPV 18, and lower than 32 for HPV Other. If this is not the case and analysis settings are correct, the experiment should be repeated.

None of the targets in the QIAscreen Negative Control should give a signal above the threshold till the end of the PCR run (i.e., cycle 40 or not defined). If a signal is seen before cycle 40, and analysis settings are correct, the experiment should be repeated.

Note: If the controls do not comply with the established limits and repetition excludes errors in technique, check the following items:

- Expiration date on reagent package
- Temperature of the reagents
- Settings of the PCR system and of the software
- Contamination

If controls are still invalid, contact the manufacturer's customer service or your local distributor.

B. Interpretation of sample results

The result for a sample is determined as follows (Table 3).

Table 3. Interpretation of results

	C_T value HPV target(s)	C_T value β-globin	Interpretation
1	HPV 16 and/or HPV 18 <36 and/or HPV Other <33.5	Any	HPV positive
2	HPV 16 and HPV 18 ≥36 or not defined and HPV Other ≥33.5 or not defined	≤30	HPV negative
3	HPV 16 and HPV 18 ≥36 or not defined and HPV Other ≥33.5 or not defined	>30	Invalid

1. **HPV positive.** When C_T value(s) of HPV 16 and/or HPV 18 is (are) <36 and/or Other HPV is <33.5 (irrespective of C_T value of β-globin). The channel indicates the type(s) present. 2. **HPV negative.** When C_T value for β-globin is ≤30 and C_T values for HPV 16 and HPV 18 are ≥36 or show no signal and HPV Other is ≥33.5 or show no signal. 3. **Invalid.** When C_T value of β-globin is >30 and C_T values of HPV 16 and HPV 18 are ≥36 or show no signal and Other HPV is ≥33.5 or show no signal.

References

1. Horner, S.M., DeFilippis, R.A., Manuelidis, L., DiMaio, D. (2004) Repression of the human papillomavirus E6 gene initiates p53-dependent, telomerase-independent senescence and apoptosis in HeLa cervical carcinoma cells. *J. Virol.* 78, 4063.
2. Butz, K., Ristriani, T., Hengstermann, A., Denk, C., Scheffner, M., Hoppe-Seyler, F. (2003) siRNA targeting of the viral E6 oncogene efficiently kills human papillomavirus-positive cancer cells. *Oncogene* 22(38), 5938.
3. Meijer, C.J., et al. (2009) Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. *Int. J. Cancer* 124(3), 516.
4. Hesselink, A. et al. (2014) Clinical validation of the HPV-Risk assay: a novel, real-time PCR assay for the detection of high-risk human papillomavirus DNA by targeting the E7 region. *J. Clin. Microbiol.* 52, 890.
5. Hiller, T., Poppelreuther, S., Stubenrauch, F., Iftner, T. (2006) Comparative analysis of 19 genital human papillomavirus types with regard to p53 degradation, immortalization, phylogeny, and epidemiologic risk classification. *Cancer Epidemiol. Biomarkers Prev.* **15**, 1262.

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 (for contact information, visit www.qiagen.com).

Comments and suggestions

Sample is scored invalid: the amplification of β -globin is too low or absent

- | | |
|--|---|
| a. Pipetting error or omitted reagents. See "PCR on Rotor-Gene Q 5 plex instruments with 72-tube rotor" on page 19 | Check pipetting scheme and the reaction setup. Repeat the sample. |
| b. Check the DNA eluate | Repeat DNA extraction. |

Positive Control is scored invalid: the amplification is too low or absent for one or more of the targets

- | | |
|--|--|
| a. Pipetting error or omitted reagents. See "PCR on Rotor-Gene Q 5 plex instruments with 72-tube rotor" on page 19 | Check pipetting scheme and the reaction setup. Repeat the sample. |
| b. Partial degradation | Store kit contents at -30°C to -15°C .
Avoid repeated freezing and thawing to a maximum of five cycles. |
| c. PCR reagents partially degraded | Store kit contents at -30°C to -15°C and keep the reaction mixes protected from light.
Avoid repeated freezing and thawing. |
| d. Strip tube inversion | Check the pipetting scheme and the reaction setup. |
| e. Expiry date | Check the expiry date of the used kit. |
| f. Time-delay between pipetting samples and start of the run | The PCR mixes can be stored 30 minutes between pipetting samples in the PCR and start of the run in the machine at $2-8^{\circ}\text{C}$ in the dark. |

No Template Control (NTC) is invalid

Comments and suggestions

a. Pipetting error or omitted reagents. See "PCR on Rotor-Gene Q 5 plex instruments with 72-tube rotor" on page 19	Check pipetting scheme and the reaction setup. Repeat the sample.
b. Cross-contamination	Replace all critical reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carryover contamination.
c. Reagents contaminated	Replace all critical reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carryover contamination.
d. Strip tube inversion	Check the pipetting scheme and the reaction setup.
e. Time-delay between pipetting samples and start of the run	The PCR mixes can be stored 30 minutes between preparation of the mixes and start of the run in the machine at 2–8°C in the dark.
f. Probe degradation	Keep reaction mixes protected from light. Check for false positive on the fluorescence curve.









Absent of low signals in sample, but the control run is okay

a. Inhibitory effects	Always check if there are no remains of buffers during DNA extraction. Repeat DNA extraction.
b. Pipetting error or omitted reagents. See "PCR on Rotor-Gene Q 5 plex instruments with 72-tube rotor" on page 19	Check pipetting scheme and the reaction setup. Repeat the PCR run.

If the problem persists, contact QIAGEN Technical Service.

Symbols

The following symbols may appear on the packaging and labeling:

Symbol	Symbol definition
	Use by
	Catalog number
	Lot number
	Material number
	Components
	Contains
	Number
Rn	R is for revision of the Instructions for Use (Handbook) and n is the revision number
	Temperature limitation

Symbol

Symbol definition



Manufacturer



Keep away from sunlight



Consult instructions for use



Global Trade Item Number



Caution

Contact Information

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
QIAscreen HPV PCR Test RUO	For 72 reactions, includes: Master Mix, Positive Control, Negative Control, Instructions for Use	617006
QIASymphony SP	QIASymphony sample prep module (optional for extraction)	9001297
Rotor-Gene Q MDx		
Rotor-Gene Q MDx HRM System	Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002035
Rotor-Gene Q MDx 5plex HRM Platform	Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002032
Rotor-Gene Q Accessories		
Loading Block 72 x 0.1 mL Tubes	Aluminum block for manual reaction set up with a single-channel pipet in 72 x 0.1 mL tubes	9018901
Strip Tubes and Caps, 0.1 mL (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1 mL (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106

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Document Revision History

Date	Changes
July 2019	Initial release
April 2024	<ul style="list-style-type: none">• Updated manufacturer address• Updated the specimen storage and handling section• Updated the sample preparation section with pretreatment of samples stored in SurePath and instructions for DNA extraction with the QIAamp DSP Virus Spin kit and DNA extraction with the QIAasymphony using the QIAasymphony DSP Virus/Pathogen Midi kit.

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