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Manual

MutaPLEX®
HPV HR screen

Real-Time-PCR-Kit

Test for the detection of high-risk human papillomavirus (HR HPV) DNA in biological specimens

Valid from 2020-07-10

REF **KSR191196**
KSR1911-384

Σ 96/384

+2°C +8°C

RUO

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Table of Contents

1 INTENDED USE

The MutaPLEX® HPV HR screen Real-Time-PCR kit is intended for the detection of high-risk human papillomavirus (HR HPV) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 DNA extracted from biological specimens. For research use only. Not for use in diagnostic procedures.

2 PATHOGEN INFORMATION

Papillomaviridae is a family of non-enveloped DNA viruses whose members are known as papillomaviruses. [1] Several hundred species of papillomaviruses, traditionally referred to as "types", [2] have been identified infecting all carefully inspected mammals, [2] but also other vertebrates such as birds, snakes, turtles and fish. [3], [4], [5]

Over 170 human papillomavirus types have been completely sequenced. [6] They have been divided into 5 genera: Alphapapillomavirus, Betapapillomavirus, Gammapapillomavirus, Mupapillomavirus and Nupapillomavirus. At least 200 additional viruses have been identified that await sequencing and classification.

3 PRINCIPLE OF THE TEST

The MutaPLEX® HPV HR screen Real-Time-PCR kit contains specific primers and dual-labelled probes for the amplification of pathogen DNA fragments.

Furthermore, MutaPLEX® HPV HR screen Real-Time-PCR Kit contains an internal control sample (IC), which is added during DNA extraction and detected in the same reaction by a FAM labelled probe.

The IC allows the detection of PCR inhibition and acts as control, that the nucleic acid was isolated from the biological specimen.

4 PACKAGE CONTENTS

The reagents supplied are sufficient for 96 (KSR191196) or 384 (KSR1911-384) reactions, respectively.

Table 1: Components of MutaPLEX® *HPV HR screen* Real-Time-PCR Kit .

Label	Lid Colour	Content	
		96	384
Positive Control	transparent	2 x 1 ml	8 x 1 ml
Internal Control Sample (IC)	orange	1 vial	4 vials

Label	Lid Colour	Content	
		96	384
Reconstitution solution	purple	1 x 1.2 ml	4 x 1.2 ml
Master Mix (lyophilized) 1	-	1 x 96 tubes	4 x 96 tubes
Master Mix (lyophilized) 2	-	1 x 96 tubes	4 x 96 tubes
PCR optical quality film	-	3 sheets	12 sheets

5 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- DNA isolation kit (e. g. MutaCLEAN® Mag RNA/DNA, KG1023 or KG1024)
- PCR grade water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Real-Time-PCR instrument (block type): e.g. iQ5 iCycler, CFX96/Opus96 (Bio-Rad USA) or DT-96 (DNA-Technology, Russia), QuantStudio 5 (Thermofisher, USA)
- Optional: Liquid handling system for automation

  * Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

6 TRANSPORT, STORAGE AND STABILITY

The MutaPLEX® HPV HR screen Real-Time-PCR kit is shipped on cool packs. All components must be stored at 2 - 8 °C in the dark immediately after receipt.

Protect kit components from direct sunlight during the complete test run.

After the initial opening of the tube, store PC at 2 – 8 °C for no more than 1 month or in 50 µl aliquots - 18 to - 24 °C for up to 3 months.

7 WARNINGS AND PRECAUTIONS

- Stick to the protocol described in the instructions for use.
- The MutaPLEX® HPV HR screen Real-Time-PCR must be performed by qualified personnel only.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.

- Avoid microbial and nuclease (DNase/RNase) contamination of the eluates and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification in order to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organisations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.

8 SAMPLE MATERIAL

Starting material for MutaPLEX® HPV HR screen Real-Time-PCR Kit is DNA isolated from biological specimens (cervical swabs).

9 SAMPLE PREPARATION

Commercial kits for DNA isolation such as MutaCLEAN® Mag RNA/DNA (KG1023 or KG1024) are recommended.

Important: In addition to the samples, always run a water control in your extraction. Treat this water control analogous to a sample.

Comparing the amplification of the IC in the samples to the amplification of the IC in the water control will give insights on possible inhibitions of the Real-Time-PCR. Furthermore, possible contaminations during nucleic acid extraction will be detectable.

Please note chapter 10 "Internal Control sample".

If the Real-Time-PCR is not performed immediately, store extracted nucleic acids according to the instructions given by the extraction kit's manufacturer.

10 INTERNAL CONTROL SAMPLE

An internal control sample (IC) is supplied as extraction control. This allows the user to control the DNA isolation procedure and to check for possible Real-Time-PCR inhibition.

Preparation of the internal control: Before use, the **lyophilised interal control sample (IC)** has to be reconstituted with **1 ml reconstitution solution (RECSOL)** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 15 minutes and then mix thoroughly. **Internal control** (reconstituted IC) is stable at **2 – 8 °C** for **4 weeks**.

The volume of IC that has to be added to the lysis buffer depends on the volume of DNA elution buffer used: 1.5 µl IC per 10 µl elution buffer.

The internal control sample must be added to the lysis buffer of the extraction kit.

11 REAL-TIME-PCR

11.1 Important points before starting

- Please pay attention to chapter 7 "Warnings and precautions".
- Before setting up the Real-Time-PCR familiarise yourself with the Real-Time-PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the PCR set up.
- In every PCR run, one positive control (PC) and one negative control (NC) should be included.
- Before each use, all reagents should be completely at room temperature, thoroughly mixed and centrifuged very briefly.

11.2 Preparation of controls

The controls must be diluted in PCR-grade water before it is used in the PCR. Please prepare the controls according to the following tables.

Table 2: Preparation of positive control

Component	Volume
Positive control	17 µl
Internal control	17 µl
PCR-grade water	80 µl
Total	**114 µl**

Table 3 Preparation of negative control

Component	Volume
Internal control	17 µl
PCR-grade water	97 µl
Total	**114 µl**

Add **50 µl** each to the **Master Mix (lyophilized) 1 (RMM1)** and **Master Mix (lyophilized) 2 (RMM2)** for the respective controls.

11.3 Procedure

Prior to the analysis, take the kit out of the refrigerator and keep **Master Mix (lyophilized) 1 (RMM1)** and **Master Mix (lyophilized) 2 (RMM2)** closed in the package at 18 – 25 °C for at least 30 min. Open the package and cut off the necessary number of tubes with **Master Mix (lyophilized) 1** and **Master Mix (lyophilized) 2** (counting test samples and control samples: 1 NC and 1 PC) with the scissors. Cut the tubes together with the covering film.

Attention! *Put the remaining tubes immediately back into the foil pouch, squeeze the air out and tightly close it with a clip. After the initial opening of the package, store the* ***Master Mix (lyophilized) 1 and Master Mix (lyophilized) 2 a****t 2 – 8 °C for no more than 3 months.*

- Label the tubes with RMM 1 and RMM2 for each test and control sample

Attention! *Labels should be placed on the lateral side of the tubes, leave optical film clean.*

- **Add 50 µl** of the corresponding **extracted, diluted* DNA** or prepared **positive/negative control** solution to the resprective tube with RMM1 and RMM 2 using a new pipette tip with filter. Tightly seal the tubes with PCR optical

quality film. (* ***The dilution depends on the extraction method. We recommend a dilution of 1:5 (10 µl eluate + 40 µl PCR grade water) as initial dilution.***)

Note: For more efficient mixing, it is recommended to shake the PCR plate on a plate shaker at 1,200 - 1,800 rpm for at least 1 min.

- Place the tubes into the real-time PCR cycler.

11.4 Instrument settings

For the Real-Time-PCR use the thermal profile shown in table 2.

Table 4: Real-Time-PCR thermal profile

Description	Time	Temperature	No of cycles
Hold	2 min	50 °C	1
Initial Denaturation	2 min	95 °C	1
Amplification of cDNA			50
Denaturation	10 s	94 °C	
Annealing and extension	20 s	60 °C	
	Aquisition at the end of this step		

In addition, the following specific settings must be made for the DT-96 cycler:

The measurement exposure must be adjusted. Choose the **Operation with the device** mode in the **Settings** menu, select the item **Measurement exposition**:

- **FAM** to **250**
- **HEX** and **ROX** to **1000**
- **Cy5** to **500**

Confirm that the current exposure value is saved by pressing **YES**.

Attention! The specified exposure values are applicable only for MutaPLEX® HPV HR screen Real-Time-PCR Kit and should be changed for other purposes.

12 DATA ANALYSIS

The following results can occur (table 5):

Table 5: Interpretation

Signal/C_T Values			Interpretation
ROX channel **RMM1 or RMM2**	**HEX channel** **RMM1**	**FAM channel** **RMM1 and RMM2**	
HPV HR	HPV HR	Internal Control	
positive (≤ 40)	negative (> 40)	positive or negative*	**Positive result**, the sample contains HPV HR DNA.
negative (> 40)	**positive** (≤ 40)	positive or negative*	**Positive result**, the sample contains HPV HR DNA.
positive (≤ 40)	**positive** (≤ 40)	positive or negative*	**Positive result**, the sample contains HPV HR DNA.
negative (> 40)	negative (> 40)	$\Delta C_T \leq 2$ from $(IC\ C_T)_{av}$**	**Negative result**, the sample contains no HPV HR DNA.
negative (> 40)	negative (> 40)	negative or $\Delta C_T > 2$ from $(IC\ C_T)_{av}$**	**Caution!** The real time PCR is either inhibited or errors occurred while DNA extraction.

* A strong positive signal in the ROX and/or HEX channel can inhibit the IC. In such cases the result for the IC can be neglected.

** If the IC C_T value for such sample differs from the an average IC C_T of all samples (including PC and NC) $(IC\ C_T)_{av}$ value by more than 2, the result is considered equivocal. A repeated analysis of the sample, starting from the DNA extraction step is required.

13 ASSAY VALIDATION

Negative control

The negative control must show a **positive** (i. e. exponential) amplification curve in the **FAM channel**. Neither HEX, ROX fluorescence increase im RMM1 nor ROX fluorescence increase in RMM2 should appear. If the C_T in the HEX or ROX channel is less than or equal to 40, it indicates the presence of contamination. In this case, all positive results of this individual PCR test run are considered equivocal. Actions are required to identify and eliminate the source of contamination. Repeat the analysis of all samples of this run that were determined positive. Samples that showed negative results in this run should be considered negative.

Positive control

The positive control must show a positive (i. e. exponential) amplification curve in the different channels FAM, HEX, ROX for Master mix 1 and FAM, ROX for Master mix 2.

Internal controls

For each test sample, the internal control should show a positive (i. e. exponential) amplification curve in the FAM channel.

Calculate $(IC\ C_T)_{av}$ as an average IC C_T of all samples (including PC and NC). IC C_T values that differ by more than 2 from $(IC\ C_T)_{av}$ should be ignored. Recalculate $(IC\ C_T)_{av}$ for the remaining values. If IC C_T value for HEX and ROX negative sample differs from $(IC\ C_T)_{av}$ value by more than 2, the result is considered equivocal. A repeated analysis of the sample, starting from the DNA extraction step is required.

14 LIMITATIONS OF THE METHOD

- Strict compliance with the instruction for use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR.
- Good laboratory practice is essential for proper performance of this assay.
- All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This assay must not be used on a biological specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this PCR assay.
- The presence of PCR inhibitors may cause false negative or invalid results.

15 TROUBLESHOOTING

The following troubleshooting guide is included to help you with possible problems that may arise when performing a Real-Time-PCR.

No fluorescence signal in the FAM, HEX and ROX channel of the positive control

The selected channel for analysis does not comply with the protocol

Select the FAM channel for analysis of the Internal Control specific amplification and the HEX and ROX channel for analysis of the HPV specific amplification.

Incorrect preparation of the Master Mix

Make sure the Master Mixes (lyophilized) 1 and 2 are completely solved (chapter 11.2).

Incorrect configuration of the Real-Time-PCR

Check your work steps and compare with chapter "Procedure".

The programming of the thermal profile is incorrect

Compare the thermal profile with the chapter 11.3 "Instrument Settings".

Incorrect storage conditions for one or more kit components or kit expired

Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in chapter "Transport, storage and stability".

Weak or no signal of the Internal Control and simultaneous absence of a signal in the virus-specific HEX and/or ROX channel

Real-Time-PCR conditions do not comply with the protocol

Check the Real-Time-PCR conditions (chapter 11).

Real-Time-PCR inhibited

Make sure that you use an appropriate isolation method (chapter 9 "Sample preparation") and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffer of the isolation kit has been completely removed.

Sample material not sufficient

Make sure enough sample material has been applied to the extraction. Use an appropriate isolation method (see chapter "Sample preparation") and follow the manufacturer's instructions

DNA loss during isolation process

In case the Internal Control was added before extraction, the lack of an amplification signal can indicate that the DNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol.

Incorrect storage conditions for one or more components or kit expired

Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in chapter "Transport, storage and stability".

Detection of a fluorescence signal in the ROX and/or HEX channel of the negative control

Contamination during preparation of the PCR

Repeat the Real-Time-PCR in replicates. If the result is negative in the repetition, the contamination occured when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the positive control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the Real-Time-PCR.

16 KIT PERFORMANCE

16.1 Analytical specificity

Analytical specificity of the MutaPLEX® HPV HR screen Real-Time-PCR kit is ensured by specific primers and probes. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. Additionally, cross-reactivity was evaluated using the control panel containing DNA of closely related species. No false-positive results were obtained.

16.2 Analytical sensitivity

Analytical sensitivity of HPV HR DNA detection was determined using probit analysis and confirmed on the following types of specimens: cervical swabs.

Note: Analytical sensitivity depends on the sample volume, elution volume, NA extraction method, and other factors.

17 ABBREVIATIONS AND SYMBOLS

DNA	Deoxyribonucleic acid	REF	Catalog number
PCR	Polymerase chain reaction	CONTENT	Content
MASTER MIX 1	Master Mix (lyophilized) 1 (RMM1)		Contains sufficient for <n> test
MASTER MIX 2	Master Mix (lyophilized) 2 (RMM2)		Upper limit of temperature
CONTROL +	Positive control		Manufacturer
CONTROL –	Negative control	LOT	Lot number
CONTROL DNA IC	Internal Control DNA (IC)		Use by
RECSOL	Reconstitution solution		Consult instructions for use
RUO	*reserach use only*		

18 LITERATURE

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