ScreenYu Gyn®

Basic UDI-DI: 426076785SCREENYUGYNP6

Instructions for use

REF	Σ	UDI-DI
SG001-46	Up to 46 samples	4260767852212

Please read these instructions for use carefully before using the test and follow them carefully to ensure the reliability of the results.

ScreenYu Gyn® is an in vitro diagnostic kit for the qualitative detection of a human epigenetic marker in bisulfite-converted DNA from cervical samples from women with a positive HPV test result or with an abnormal Pap finding pending clarification. A positive ScreenYu Gyn® result is associated with the presence of cervical intraepithelial neoplasia or cervical cancer.

To be used for in vitro diagnostics (IVD) by trained personnel only.

Revision 6 (2025-04)
Translation released 2025-04

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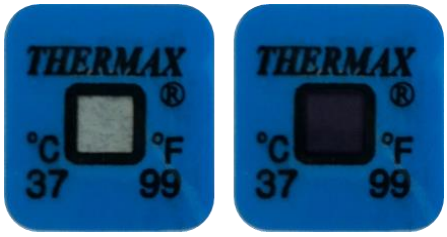
STORE IMMEDIATELY AFTER DELIVERY

The ScreenYu Gyn® Kit is shipped at room temperature and the temperature measuring mark is used to monitor whether the validated temperature interval is exceeded. Immediately after receipt, check the temperature measuring mark attached to the kit and confirm whether the colour has changed. Also check the outer packaging, seal and primary packaging and confirm they are not damaged. The kit must be refrigerated immediately upon receipt at a temperature between 2 °C and 8 °C, and protected from light. If transported and stored properly, the ScreenYu Gyn® Kit and its components can be used until the stated date.



Monitoring the transport temperature

The temperature measuring mark attached to the ScreenYu Gyn® Kit monitors temperature during transport. A light silver mark indicates that the kit was delivered in compliance with the transport temperature. A black mark proves non-compliance with the specified transport temperature, which means that the performance parameters of the ScreenYu Gyn® Kit can no longer be guaranteed. In this case, please contact oncgnostics GmbH.

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1 PURPOSE

ScreenYu Gyn® is an in vitro diagnostic kit for the qualitative detection of a human epigenetic marker in bisulfite-converted DNA from cervical samples of women with a positive HPV test result or with an abnormal Pap test finding pending clarification. A positive ScreenYu Gyn® result is associated with the presence of cervical intraepithelial neoplasia or cervical cancer.

ScreenYu Gyn® is intended for use only by qualified laboratory personnel familiar with molecular biology techniques. The interpretation of the results should always be carried out in conjunction with results of further laboratory diagnostic procedures, as well as taking into account the clinical picture.

2 CLINICAL SIGNIFICANCE

Cervical cancer is the fourth most common cancer in women worldwide, with > 600,000 new cases annually [1]. In virtually all cases, persistent infection with a carcinogenic human papillomavirus (HPV) is the underlying cause [2] and a prerequisite for the development of cervical cancer. HPV-negative women have an extremely low risk of developing cervical cancer, but even most women with an HPV infection do not develop a precancerous stage. Only about 15% of women infected with HPV actually develop a precancerous stage or carcinoma that requires treatment [3].
Patients with a positive HPV test result and/or with Pap findings pending clarification (Pap II, Pap III and Pap IIID1 and D2) are therefore recommended to use a triage test such as ScreenYu Gyn®, to determine the probability of the presence of a cancer or its precursor with high accuracy.
ScreenYu Gyn® should not be considered as the final therapeutic decision and must be analysed in conjunction with other medical findings.

3 TEST PRINCIPLE

ScreenYu Gyn® is based on the detection of an epigenetic biomarker, i.e., the methylation of a specific DNA section, the presence of which corresponds to the presence of precancerous lesions or cervical cancer [4, 5, 6]. In addition, a bisulfite-specific reference marker is analysed. The marker regions used are shown in the table below.

Overview of marker regions

Designation in the protocol	Marker region (gene designation)	Fluorescent dye
Methylation marker	ZNF671	ROX
Control marker	ACTB	FAM

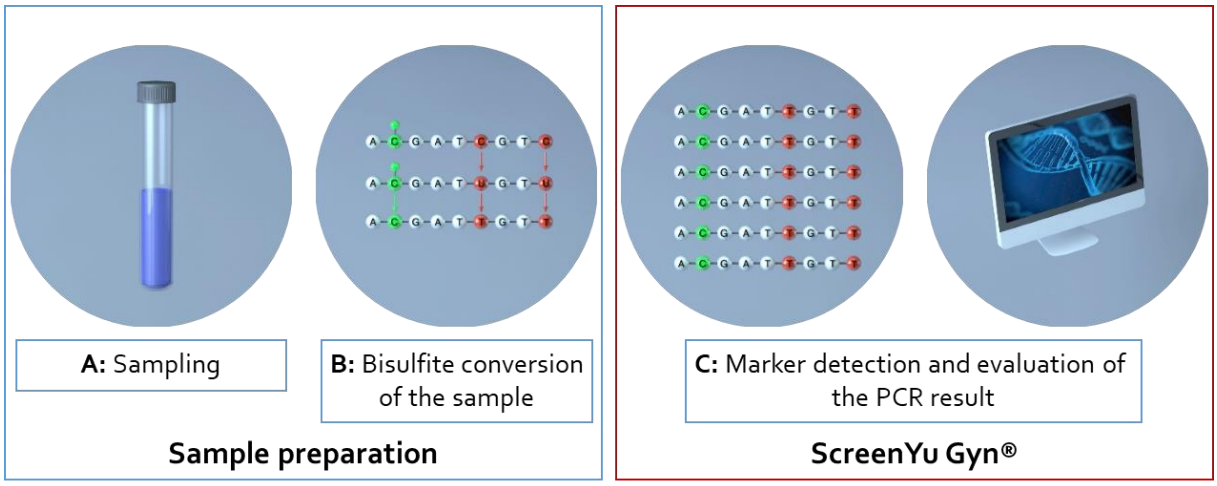
Detection is performed using the highly sensitive probe-based Real-Time PCR method. The output of the Real-Time instruments is the Cp value (Cross point, cobas z 480 Analyzer) or Cq value (Cycle quantification, CFX96 Real-Time PCR Detection System), both of which correspond to the Cycle threshold Ct value and are also referred to as such below. This value corresponds to the cycle in a Real-Time PCR in which fluorescence rises above a defined threshold value for the first time.

The analysis of a patient sample using ScreenYu Gyn® involves two steps.

First, DNA from the cervical smear is converted by bisulfite and methylation is fixed. In the second step, the eluted, bisulfite-treated DNA is analysed in a methylation-specific, probe-based Real-Time PCR. The originally methylated DNA section is selectively amplified using the primers in the ScreenYu Gyn® Strips. The detection of the methylation and control marker is performed using

probes labelled with fluorescent dyes. In addition, a positive and a negative control are included to confirm the PCR. Subsequently, the assay-specific analysis is performed.

The Sampling and the bisulfite Kit are not part of the ScreenYu Gyn® Kit. Products specifically designed for sampling and bisulfite treatment are available separately.



Test principle

***A**: The gynaecologist takes a smear from the patient's cervix using a suitable sampling kit.*
***B**: The diagnostic laboratory performs bisulfite treatment on the patient sample.*
***C:** One duplex-PCR reaction is performed per sample. The analysis is performed by detecting the dye-labelled probes contained in the ScreenYu Gyn® Strip.*

4 ScreenYu Gyn® Assay Design

4.1 ScreenYu Gyn® Strips

ScreenYu Gyn® is a TaqMan probe-based assay. A ScreenYu Gyn® Strip is an 8-well PCR strip containing two primer pairs in each well, as well as an associated probe for amplification of the methylation-specific marker ZNF671 and the control marker ACTB. One well of a ScreenYu Gyn® Strip is required for the analysis of a patient sample.

4.2 Controls

The design of the ScreenYu Gyn® Kit includes three controls to monitor the sample quality and the bisulfite treatment (ACTB marker) as well as the quality of the PCR reaction (positive control and negative control).

4.2.1 Quality control bisulfite treatment (control marker ACTB)

This control marker verifies the successful conversion of all non-methylated cytosines to uracil and thus the quality of the bisulfite treatment performed. Detection is performed by amplification of a DNA fragment close to the human gene beta-Actin (ACTB). If there is no ACTB amplification with a Ct value below 32, the assay of the sample is considered invalid and must be repeated.

4.2.2 Positive control

The positive control monitors the quality of the PCR. The amplification of the ScreenYu Gyn® Positive Control (PC) should provide a Ct value below 38 for both the methylation marker and the control marker. Otherwise, the PCR is invalid and must be repeated.

4.2.3 Negative control

The negative control is a control reaction with ScreenYu Gyn® Water (NTC – No Template Control) as a template, which must be negative in both markers. If Ct values occur in the negative control, contamination is very likely and the ScreenYu Gyn® assay must be repeated.

5 REFERENCE MATERIAL

No international reference material is available.

6 KIT CONTENTS

Contents of the ScreenYu Gyn® Kit

Designation of components	Symbol	Content	Volume/Quantity SG001-46
ScreenYu Gyn® Mastermix	PCR-MM	PCR Mastermix [1] (2x)	1 x 0.55 ml
ScreenYu Gyn® Strips	STRIPS	PCR strip [2]	6 Strips
ScreenYu Gyn® Caps	CAPS	Cap for Strip	6 Caps
ScreenYu Gyn® Positive Control	CONTROL +	Positive control	1 x 90 µl
ScreenYu Gyn® Water	H_2O	Water	1 x 2 ml
Instructions for use	-	Instructions for use	1

[1] Contains all components required for the polymerase chain reaction (PCR), except primers, probes and template.
[2] Contains the primers and probes required for PCR.

7 CONSUMABLES AND EQUIPMENT (NOT INCLUDED IN THE KIT)

ScreenYu Gyn® may only be used together with the listed consumables and equipment and only by qualified personnel. All required laboratory equipment must be installed, calibrated, handled and maintained according to the manufacturer's instructions.
Room temperature is defined as between 15 °C and 30 °C.

Required equipment

Equipment	Catalogue no.	Company
EZ DNA Methylation-Lightning Kit (CE IVD)	D5030-E, D5031-E	Zymo Research Europe GmbH
PCR microcentrifuge tube PP, 0.1 ml, without cap, low profile, 8-well strip, white, np pcr ready *	04-032-0556	Nerbe plus GmbH & Co. KG
Cap for PCR microcentrifuge tubes PP, 0.1 ml & 0.2 ml, flat, 8-cap strip, highly transparent, np pcr ready *	04-043-0500	Nerbe plus GmbH & Co. KG
ThinPrep® PreservCyt® Solution (20 ml)	-	Hologic, Inc.
Cervex-Brush® or Cervex-Brush® Combi	-	Rovers Medical Devices

* To be used as Balance Strips in PCR, see plate layout on page 15 or page 19.

The following laboratory equipment and consumables are required to perform the ScreenYu Gyn® assay.

- Centrifuge for 0.5 ml/1.5 ml reaction tubes, ≥ 10,000 xg
- Centrifuge for PCR strips
- Thermal cycler for 0.5 ml reaction tubes
- Vortex mixer / shaker
- Pipettes with different volume ranges and associated filter tips (sterile, DNase-free)
- Reaction tube stand for 0.5 ml/1.5 ml/2 ml reaction tubes
- 96-well rack for PCR strips
- Reaction tubes for 0.5 ml/1.5 ml (DNase-free)
- Ethanol 96 – 100%, undenatured
- Real-time PCR device, detection channels for probe dyes FAM and ROX
  ScreenYu Gyn® has been validated on the following real-time PCR devices:
  - cobas z 480 Analyzer (Roche Diagnostics GmbH) with 96-well-block, adapter for PCR strips and LightCycler® 480 Software UDF 2.0.0 (Service Pack 3), evaluation with version 1.5.1.62
  - CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) with CFX Maestro Software version 2.3

8 STORAGE AND SHELF-LIFE

If transported and stored properly, the ScreenYu Gyn® Kit and its components can be used until the stated date. All reagents contained in the kit are stable until the indicated expiry date after opening, if stored under the indicated conditions and protected against contamination.

Storage temperature of the ScreenYu Gyn® Kit and equipment not included in the kit

Equipment	Storage temperature
ScreenYu Gyn® Kit	2 °C to 8 °C
EZ DNA Methylation-Lightning Kit (CE IVD)	15 °C to 30 °C
ThinPrep® PreservCyt® Solution (20 ml)	15 °C to 30 °C
Cervex-Brush® or Cervex-Brush® Combi	15 °C to 30 °C

9 SAFETY INSTRUCTIONS

9.1 General information

When establishing state-of-the-art molecular biology methods, the instructions below must be followed closely to ensure maximum safety for laboratory personnel and to achieve high-quality results:

- As it involves molecular biology processes, such as bisulfite treatment, amplification, and the detection of DNA, this kit is intended only for in vitro diagnostics and should be used only by personnel trained in laboratory practices for in vitro diagnostic.
- Before using the product, read the instructions for use thoroughly. Only the current version is to be taken into account.
- Wear a suitable lab coat, disposable gloves and, if necessary, safety goggles for each step.
- Avoid direct contact with the biological samples, as well as splashing or spraying of the samples.
- The heated lid and incubation block of the thermal cycler can reach temperatures of up to 110 °C. There is a risk of skin burns. Please observe the operating instructions of the device.
- Wash your hands thoroughly after handling samples and reagents.
- Do not use ScreenYu Gyn® if the reagent packaging is damaged. Contact your distributor.
- Do not use the ScreenYu Gyn® Kit after the expiry date and do not use expired reagents.
- Do not mix reagents from different batches and do not mix kit reagents with reagents from other manufacturers.
- Use only materials supplied with the kit or recommended by the manufacturer.
- All required laboratory equipment must be installed, calibrated, handled and maintained according to the manufacturer's instructions.
- Pipetting small volumes of liquid within the microlitre range requires practice. Make sure you pipette the required volumes with the micropipettes as precisely as possible.
- The applicable regulatory requirements for the operator must be complied with.
- Adherence to Good Laboratory Practice (GLP) as outlined, for example, by the U.S. Food and Drug Administration (FDA) or the Organisation for Economic Co-operation and Development (OECD) is assumed. Specifically, recommendations for performing molecular amplification testing should be considered.

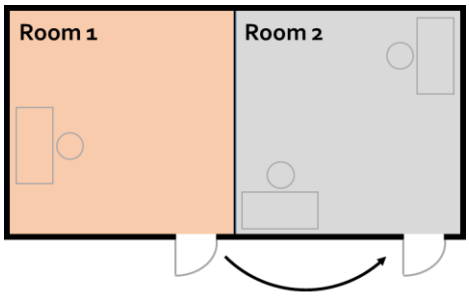
- The proper functioning of the PCR devices is only guaranteed at room temperature.

9.2 Room layout

Due to the high analytical sensitivity of PCR, strict attention should be paid to maintaining the purity of the kit components and samples.

PCR multiplies sections of the DNA in the sample millions to billions of times. Even the smallest amounts of these PCR products (e.g. also spread as aerosol) can lead to a false result if they are carried over into the sample material, into the reagents for bisulfite treatment or into the PCR reagents of this kit.

A clean and well-structured workflow is therefore crucial to prevent incorrect results. To this end, it is necessary to separate the laboratory areas for pre-PCR and post-PCR from each other. Separate equipment, consumables, lab coats and gloves should be available in each area. Never transfer lab coats, gloves or equipment from one area to the other. The figure below shows an example of a laboratory divided into two separate rooms. One area is designated only for bisulfite treatment and preparation of PCR, while in the other area the PCR is carried out.



Spatial division

The bisulfite treatment of the samples as well as the entire PCR preparation are performed in Room 1 (the use of a PCR Hood is optimal). In Room 2, the PCR is carried out, detected and analysed.

9.3 Avoiding contamination

- Lab coats and disposable gloves must be worn during all steps.
- Disposable gloves should be changed frequently and always after (suspected) contamination with reagents or sample material.
- All surfaces, equipment, and supplies must be decontaminated with a suitable cleaning solution (DNA-destroying agents).
- Do not touch the inside of the reaction tubes or their caps.
- When pipetting, filter tips (free of DNase, RNase and human DNA) must always be used to exclude cross-contamination via aerosols generated during pipetting. Tips should always be changed between pipetting steps.
- It is important to perform negative controls to detect possible contamination.

9.4 Handling instructions

- Store the unused components in the original packaging until used.
- All centrifugation steps should be performed at room temperature.
- The workflow can be interrupted after the bisulfite treatment. At this point, the samples can be stored for one week at 2 °C to 8 °C or up to two months at – 15 °C to – 30 °C.
- The ScreenYu Gyn® Strips and ScreenYu Gyn® Caps should not be touched without disposable gloves throughout the entire procedure, otherwise non-specific fluorescence signals may occur.

- The ScreenYu Gyn® Strips and ScreenYu Gyn® Caps are intended for single use and cannot be reused.
- Keep the unused ScreenYu Gyn® Strips and ScreenYu Gyn® Caps in their original packaging. It is imperative that you keep the ScreenYu Gyn® Strips away from light.

10 DISPOSAL

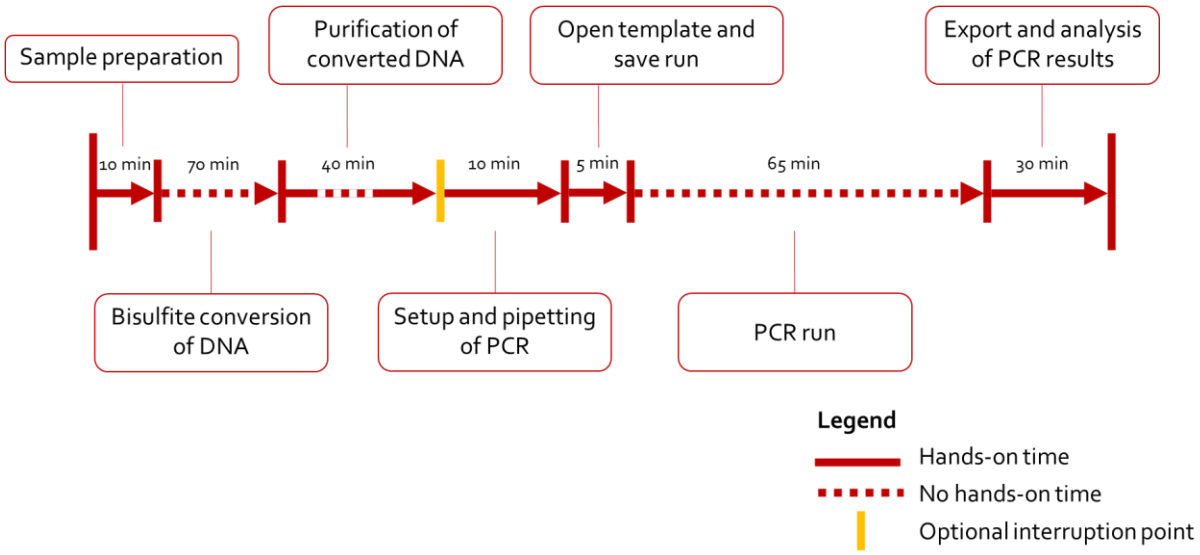
The unused ScreenYu Gyn® Kit and its components can be disposed of without further special precautions. Patient samples and used reaction tubes must be handled as infectious waste. All reagents must be disposed of in accordance with legal regulations.

11 SCREENYU GYN® PROCEDURE

The following chapter contains a detailed description of the different steps from sampling to data analysis.

11.1 Workflow

In total, ScreenYu Gyn® can be performed in less than four hours. The active working time is approximately 2 hours. During the initial ScreenYu Gyn® procedure, 15 minutes should be allowed for the creation of the PCR template.



Timeline of the ScreenYu Gyn® workflow

11.2 Sampling

The sampling kit is not included in the ScreenYu Gyn® Kit. ThinPrep® PreservCyt® vials (Hologic, Inc.) and Cervex-Brush® sampling devices (Rovers Medical Devices) are available by contacting the respective manufacturers. The collection of a cervical sample by the physician is to be performed in accordance with the manufacturer's instructions and in compliance with the generally accepted guidelines for the collection of a cervical smear sample [7].

Important: The brush head of the sample collection device should not remain in the sample container after collection, otherwise the performance of ScreenYu Gyn® will be impaired.

ThinPrep® PreservCyt® Solution must be used as the smear medium. The use of other sample media was not part of the validation of the ScreenYu Gyn® assay.

Ensuring the good quality of the employed DNA sample is an important prerequisite for the validity of the assay. Improper sampling, bisulfite treatment, and DNA storage may lead to invalid or even false negative results.

Cervical samples can be transported to the laboratory for testing without refrigeration. Samples can be stored for up to 1.5 years at temperature 2 °C to 30 °C.

11.3 Sample preparation

The following steps must be carried out in the sample preparation area (Room 1).

Important: If the brush head of the swab collection brush is inside the sample vial, it must first be removed and discarded.

- Vortex all patient samples for 5 seconds at maximum speed and immediately transfer 1 ml of the medium to a 1.5 ml reaction tube.

  Caution: The cells settle back to the bottom of the tube very quickly. No more than 10 seconds should elapse between mixing the patient sample and taking the 1 ml sample!

- Centrifuge the samples for **5 minutes** at **10,000 xg**.
- Carefully remove 900 µl supernatant above the pellet without destroying the pellet.

  Caution: Depending on the nature of the sample, the pellet is more or less solid.

- Resuspend the pellet by vortexing for 3 seconds. Add 20 µl of the resuspended sample to the bisulfite treatment. Discard the remaining 80 µl.

11.4 Bisulfite treatment of samples

The bisulfite kit is not included in the ScreenYu Gyn® Kit. ScreenYu Gyn® was validated with the EZ DNA Methylation-Lightning Kit (Zymo Research Europe GmbH).

- **Input:** Add **20 µl** of the **resuspended sample** + **130 µl of Lightning Conversion Reagent** to a 0.5 ml reaction tube.
- perform bisulfite treatment according to the manufacturer's instructions of the EZ DNA Methylation-Lightning Kit, except for the following modifications:
  - Discard the flow through after the last wash step.
  - Centrifuge the column in the empty Collection Tube for **1 minute** at **full speed** to dry it completely.

    Attention: Do not skip this step, since residual Ethanol may impair performance of the ScreenYu Gyn® assay.

  - Elute in **15 µl of M-Elution Buffer** for **30 seconds** at **8,000 xg.**

11.5 PCR

Before starting the PCR, ensure that the PCR temperature protocol is programmed into the appropriate real-time PCR device to minimise the time between preparation and PCR start. To establish the PCR programme on the cobas z 480 Analyzer, proceed as described on page 14. The explanation for PCR on the CFX96 Real-Time PCR Detection System can be found on page 19 onwards.

11.5.1 Preparation and pipetting of the PCR

Important: PCR preparation and pipetting should not take longer than 60 minutes. This step is performed in Room 1 (pre-PCR area).

Please note the plate layout described on page 15 or 19 respectively. The positive control (PC) must be pipetted in well A1 and the negative control (NTC) in well B1.

- Remove the **ScreenYu Gyn® Mastermix** and the required number of **ScreenYu Gyn® Strips** from the kit and place them on a 96-well rack.

  Caution: One **ScreenYu Gyn® Strip** is sufficient for eight PCR assays. Please note that a positive control and a negative control (water, NTC) must be carried out for each PCR run.

- Vortex the **ScreenYu Gyn® Mastermix** for 3 seconds at maximum speed and centrifuge it off.
- Remove the **PCR caps** from the ScreenYu Gyn® Strips and **discard** them.
- Add **10 µl** of **ScreenYu Gyn® Mastermix** to each well of the **ScreenYu Gyn® Strip**.
- Vortex the sample eluate for 3 seconds at maximum speed and centrifuge briefly.
- Add **10 µl of the sample** to the well filled with **ScreenYu Gyn® Mastermix.**

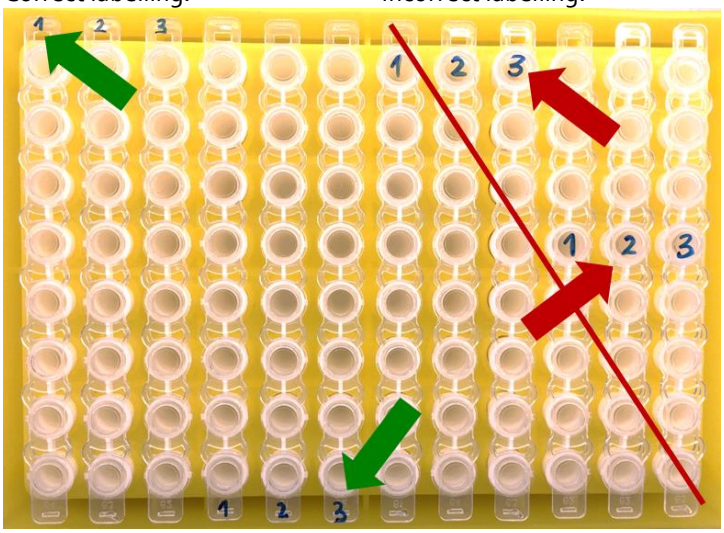
  Important: Change the pipette tip for each pipetting step.

  Note: Keep the rest of the sample eluates for a repetition of the ScreenYu Gyn® PCR if necessary.

- Vortex the **ScreenYu Gyn® Positive Control** for 3 seconds at maximum speed and centrifuge it off.
- Add **10 µl of ScreenYu Gyn® Positive Control** to well A1 and **10 µl of ScreenYu Gyn® Water** to well B1 as a negative control.
- Close each **ScreenYu Gyn® Strip** with an unused **ScreenYu Gyn® Cap** (transparent bag).

  Caution: Do not touch the inside of the **ScreenYu Gyn® Strips** and **ScreenYu Gyn® Caps**. Make sure that the **ScreenYu Gyn® Cap** is properly seated on the **ScreenYu Gyn® Strip** after closing. Verification is best carried out via visual inspection from the side.

Correct labelling: Incorrect labelling:



Caution:
Do not label the part of the caps that is directly above the wells, as the PCR signal is read from above through the caps and this would thus lead to incorrect fluorescence signals.

The large latch at the top and bottom of the cap can be used for labelling.

- Vortex all closed **ScreenYu Gyn® Strips** for 3 seconds at maximum speed and centrifuge them off.

11.5.2 Performing the PCR on the cobas z 480 Analyzer

The following section outlines how to perform the ScreenYu Gyn® on the Real-Time PCR systems cobas z 480 Analyzer (marked blue) and CFX96 Real-Time PCR Detection System (marked green). However, always follow the manufacturer's instructions for operating PCR devices.

The PCR is to be carried out in Room 2.

11.5.2.1 Creating a PCR template

If you created and saved the PCR template earlier, you can continue with 11.5.2.2 *Starting the PCR run*.

- Turn on the cobas z 480 Analyzer and its computer. Within 15 seconds, select "User defined Workflow" on the computer screen in the BIOS to switch to a freely programmable device mode.
- Open the software and log in.
- Select "Tools"  in the action bar on the right and create a new *Detection Format*. Name it ScreenYu Gyn. Select the filter combination 465-510 and 540-610 (Excitation-Emission). Close the window by clicking the *Close* button.
- Select *New Experiment* to create a new template.
  In the tab *Run Protocol*, set the *Detection Format* to *ScreenYu Gyn* and set the *Reaction Volume* to 20 µl. Program the temperature protocol according to the table below.

PCR temperature protocol on the cobas z 480 Analyzer

Programme Name	Number of cycles	Analysis Mode	Target	Acquisition Mode	Hold (hh:mm:ss)	Ramp rate (°C/s)
Initialization	1 x	None	94 °C	None	00:01:00	4.4
Amplification	42 x	Quantification	94 °C	None	00:00:15	4.4
			61 °C	Single	00:00:30	2.2
Cooling	1 x	None	37 °C	None	00:01:00	2.2

- Save the run template under the name ScreenYu Gyn by selecting *Apply Template* → *Save as Template* and storing the template in the desired location.

11.5.2.2 Starting the PCR run

If you saved the PCR template earlier, you can now access it by clicking on *New Experiment from Template*→ *ScreenYu Gyn.* Check that the correct temperature protocol is set.

- Depending on the plate layout, select a *Subset Template* in the *Subset Editor*. To do this, press the "+" button, select all occupied wells in the layout and confirm the template with the *Apply* button. This *Subset Template* can be saved at the desired location via *Apply Template* → *Save as Template.*
- Select a suitable plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip
B	NTC											
C	1											
D	2											
E	3											
F	4											
G	5											
H	6											

Example of plate layout for 6 patient samples (1 – 6)

Analysis of an incompletely occupied plate is performed by means of a defined subset in the Subset Editor.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	7	15	23	31	39	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip
B	NTC	8	16	24	32	40						
C	1	9	17	25	33	41						
D	2	10	18	26	34	42						
E	3	11	19	27	35	43						
F	4	12	20	28	36	44						
G	5	13	21	29	37	45						
H	6	14	22	30	38	46						

Example of plate layout for 46 patient samples (1 – 46)

Analysis of an incompletely occupied plate is performed by means of a defined subset in the Subset Editor.

Important: The plate layout is **not** variable. The positive control (PC) must be positioned in well A1 and the negative control (NTC) in well B1.
Empty positions should be filled with empty strips (Balance Strips) for balancing, see Chapter 7.

- Define the sample labelling in the *Sample Editor* by selecting a defined subset template at *Step 2: Select Samples* → *Subset* and entering the *Sample Name* at *Step 3: Edit Properties*. This sample template can be saved at the desired location via *Apply Template* → *Save as Template*.
- Place the strips vertically into the PCR device in the defined order.

  Important: Use the adapter for PCR strips (by Roche Diagnostics GmbH).
- Save the PCR run under a unique name in the desired folder by pressing the "floppy disk" button (on the right side of the action bar) and start the PCR run by clicking on the button *Start Run* in the tab *Run Protocol* (*Experiment Editor*).

11.5.2.3 Exporting the data

If you are analysing the PCR run directly on the cobas z 480 Analyzer computer, please continue with *11.5.2.4 Analysing the PCR data*.

- After completion of the PCR *(Run complete)*, export the PCR run via  "Export" and save the file to the desired location.

11.5.2.4 Analysing the PCR data

The following describes the analysis of the exported data. These instructions have been created using the Microsoft Excel spreadsheet programme. It is also possible to use other suitable programmes.

- If you have exported the PCR run, start the LightCycler® 480 software on another computer and open/import the PCR run. Otherwise, perform the analysis on the computer of the cobas z 480 Analyzer.
- Under *Analysis*, select the analysis algorithm *Abs Quant/Fit Points* and the specified subset, if necessary.
- In the tab *Cycle Range*, set the following parameters: *First Cycle* 1, *Last Cycle* 42 and the *Background* to 5 to 20, by setting a *Min Offset* of 4 and a *Max Offset* of 19.
- In the *Noise Band* tab, check that the *STD Multiplier* is set to 12 and the *Noise Band* is calculated automatically.
- Due to the detection of two different fluorescent dyes, the analysis of the PCR data is performed separately for each marker. The respective detection channel is selected via the button *Filter Comb*:

Filter combinations and threshold settings on the cobas z 480 Analyzer

Marker	Detection channel	Probe	Threshold
ACTB	465 – 510	FAM	1.2
ZNF671	540 – 610	ROX	0.5

- In the *Analysis* tab, check that the number of *Fit Points* is set to 2.
- Set the *Threshold* for **ACTB** to **1.2** and press the *Calculate* button to perform the analysis. Export the data table as a .txt file by right-clicking *Export Table* under *Samples* and save it in a suitable location under a unique name.
- Then set the *Threshold* for **ZNF671** to **0.5** and press the *Calculate* button to perform the analysis. Export the data table as a .txt file by right-clicking *Export Table* under *Samples* and saving it in a suitable location under a unique name.

Caution: Always analyse the two markers one after the other as described, since the programme always applies manually-set threshold values to all filters.

- Open a spreadsheet programme such as Microsoft Excel and copy all data from both .txt files, both for ZNF671 marker (Selected Filter: 540-610) and for the ACTB marker (Selected Filter: 465-510), into it.

- Format the data so that the results of the different samples are displayed one below the other and the ZNF671 and ACTB markers are displayed side by side.

Pos	Name	Cp $_{ZNF671}$	Cp $_{ACTB}$	ΔCp $_{ZNF671-ACTB}$
A1	PC	31.98	32.12	
B1	NTC			
C1	Sample 1		36.22	
D1	Sample 2	36.39	30.22	6.17
E1	Sample 3		31.50	
F1	Sample 4		31.27	
G1	Sample 5	31.61	30.32	1.29
H1	Sample 6		31.21	

Checking the validity of the PCR run

The PCR run is valid if the positive and negative control meet the following criteria:

Validity criteria of the ScreenYu Gyn® controls

Marker	Cp value for positive control	Cp value for negative control
ZNF671	≥ 20; ≤ 38	no value
ACTB	≥ 20; ≤ 38	no value

Checking the validity of the samples

The result of the patient sample is valid if the control marker ACTB meets the following criterion:

Validity criteria of the patient sample

Marker	Cp value for patient sample
ACTB	≥ 20; ≤ 32

Analysis of the ScreenYu Gyn® Assay

If the methylation marker ZNF671

- Does not yield a Cp value, the **ScreenYu Gyn® result** for this sample will be considered **negative.**
- Yields a Cp value > 0; < 20, the **ScreenYu Gyn® result** for this sample will be considered **invalid**.
- Yields a Cp value ≥ 20; ≤ 42, the ΔCp is calculated according to the following equation:

Calculation of ΔCp
$\Delta Cp = Cp_{ZNF671} - Cp_{ACTB}$

If **ΔCp ≤ 9.00,** the **ScreenYu Gyn® result** for this sample will be considered **positive**.

If **ΔCp > 9.00,** the **ScreenYu Gyn® result** for this sample will be considered **negative**.

A positive ScreenYu Gyn® result is associated with the presence of cervical intraepithelial neoplasia or cervical cancer. ScreenYu Gyn® should not be considered as the final therapeutic decision and must be analysed in conjunction with other medical findings.

11.5.3 Performing the PCR on the CFX96 Real-Time PCR Detection System

11.5.3.1 Creating a PCR template

- Switch on the PCR device.
- Program the PCR temperature protocol as described in the table below by selecting and editing the temperature steps and times.

PCR temperature protocol** on the CFX96 Real-Time PCR Detection System

Programme Name	Step	Number of cycles	Temperature	Time (m:ss)
Initialization	1	1 x	94 °C	1:00
Amplification	2	42 x	94 °C	0:15
	3*		61 °C	0:30
	4		GO TO Step 2	41 x
Cooling	5	1 x	30 °C	1:00

* *The fluorescence signal is detected via "Plate Read" during Step 3, which is symbolised by the camera symbol.*

** *On the CFX96 Real-Time PCR Detection System, the default ramp rate is 5 °C/sec. This setting was used to validate this IVD test.*

- Set the reaction volume to 20 µl and the temperature of the Lid heater to 105 °C.
- Save the PCR template using the name ScreenYu Gyn.

11.5.3.2 Starting the PCR run

If you saved the PCR template earlier, you can now access it. Check that the correct temperature protocol is set.

- Place the **ScreenYu Gyn® Strips** into the PCR device by inserting them vertically into the small wells of the heating block. Select a suitable plate layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip
B	NTC											
C	1											
D	2											
E	3											
F	4											
G	5											
H	6											

Example of plate layout for 6 patient samples (1 - 6)

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	7	15	23	31	39	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip	Balance Strip
B	NTC	8	16	24	32	40						
C	1	9	17	25	33	41						
D	2	10	18	26	34	42						
E	3	11	19	27	35	43						
F	4	12	20	28	36	44						
G	7	13	21	29	37	45						
H	6	14	22	30	38	46						

Example of plate layout for 46 patient samples (1 - 46)

Important: The plate layout is **not** variable. The positive control (PC) must be positioned in well A1 and the negative control (NTC) in well B1.
Empty positions should be filled with empty strips (Balance Strips) for balancing, see Chapter 7.

- Name the run using a suitable file name. Note that *All Channels* are detected before you start the run.

11.5.3.3 Exporting the data

- After completing the PCR, export the PCR run (.pcrd file).

11.5.3.4 Analysing the PCR data

The following describes the analysis of the exported data.

- On a computer, open the BioRad CFX Software and first set *Plate Type: BR White* under *User* → *User Preferences* → *Plate* to indicate that white plastic is used.
- Import the .pcrd file.
- Define the plate layout under *Plate Setup* → *View/Edit Plate*. Click on the *Select Fluorophores* button to select the employed dyes FAM and ROX. The corresponding markers ACTB (FAM) and ZNF671 (ROX) can be selected under *Target Names*. Enter the name under *Sample Names*.
- Unoccupied positions are marked and can be excluded from the analysis by checkmarking *Exclude Wells in Analysis*.
- Confirm the plate layout under *OK*.
- Set the analysis settings under *Settings*. The parameters under *Baseline Threshold* must be defined separately for each fluorophore. To this end, the markers in the *Quantification* tab must be deselected or selected.

Analysis settings on the CFX96 Real-Time Detection System

Parameter	Setting
Cq Determination Mode	Single Threshold
Baseline Setting	Baseline Subtracted Curve Fit
	Apply Fluorescence Drift Correction
Analysis Mode	Target
Cycles to Analyze	1-42
Baseline Threshold	Baseline Cycles → User Defined: Begin: 5; End: 20
	Single Threshold → User Defined: 200

- Select all samples and export the data for both markers at the same time as an .xlsx file in a suitable location under a unique name by right-clicking on *Export to Excel*.

- Format the data so that the results of the different samples are displayed one below the other and the ZNF671 and ACTB markers are displayed side by side.

Pos	Name	Cq_{ZNF671}	Cq_{ACTB}	$\Delta Cq_{ZNF671-ACTB}$
A1	PC	30.32	30.33	
B1	NTC			
C1	Sample 1		34.16	
D1	Sample 2	35.74	29.23	6.50
E1	Sample 3		29.60	
F1	Sample 4		29.47	
G1	Sample 5	30.61	28.48	2.13
H1	Sample 6		28.72	

Checking the validity of the PCR run

The PCR run is valid if the positive and negative controls meet the following criteria:

Validity criteria of the ScreenYu Gyn® controls

Marker	Cq value for positive control	Cq value for negative control
ZNF671	≥ 20; ≤ 38	no value
ACTB	≥ 20; ≤ 38	no value

Checking the validity of the samples

The result of the patient sample is valid if the control marker ACTB meets the following criterion:

Validity criteria of the patient sample

Marker	Cq value for patient sample
ACTB	≥ 20; ≤ 32

Analysis of the ScreenYu Gyn® Assay

If the methylation marker ZNF671

- Does not yield a Cq value, the **ScreenYu Gyn® result** for this sample will be considered **negative**.
- Yields a Cq value > 0; < 20, the **ScreenYu Gyn® result** for this sample will be considered **invalid**.
- Yields a Cq value ≥ 20; ≤ 42, the ΔCq is calculated according to the following equation:

Calculation of ΔCq
$\Delta Cq = Cq_{ZNF671} - Cq_{ACTB}$

If **ΔCq ≤ 10.00**, the **ScreenYu Gyn® result** for this sample will be considered **positive**.

If **ΔCq > 10.00**, the **ScreenYu Gyn® result** for this sample will be considered **negative**.

A positive ScreenYu Gyn® result is associated with the presence of cervical intraepithelial neoplasia or cervical cancer. ScreenYu Gyn® should not be considered as the final therapeutic decision and must be analysed in conjunction with other medical findings.

12 SCREENYU GYN® PERFORMANCE

The performance data of the CFX96 Touch Real-Time PCR Detection System are shown. In case of deviations of the data from the cobas z 480 Analyzer, these are listed separately. If the data from the cobas z 480 Analyzer are not explicitly shown, they correspond to the data shown for the CFX96 Touch Real-Time PCR Detection System.

12.1 Analytical performance

12.1.1 Analytical sensitivity

The analytical sensitivity of the PCR assay was determined using methylated bisulfite-converted genomic human DNA. The respective detection limits are listed in the following table. The dilution series were tested in 9-fold determination. On average, 120 - 180 ng of DNA is used in the assay for one smear.

Analytical Sensitivity – Part 1

DNA used	Approximate number of cells in the assay*	ZNF671 Cq ≤ 42	ACTB Cq ≤ 42
0.2 ng	30 cells	9 / 9	9 / 9
0.1 ng	15 cells	9 / 9	9 / 9
0.05 ng	7.5 cells	9 / 9	9 / 9
0.02 ng	3 cells	9 / 9	9 / 9
0.01 ng	1.5 cells	9 / 9	7 / 9
0.005 ng	< 1 cell	5 / 9	3 / 9

* *one cell contains approx. 6 – 7 pg genomic DNA*

The detection limit for the two markers on the CFX96 Touch Real-Time PCR Detection System is a total of 3 cells (0.02 ng) in the total sample.

On the cobas z 480 Analyzer, the detection limit for the two markers is a total of 7.5 cells (0.05 ng) in the total sample.

The quantification limits of the ScreenYu Gyn® assay correspond to the detection limits, resulting in a linearity of $R^2 = 0.99$ for both markers within the quantification limits.

In addition, a DNA mixture of methylated bisulfite-converted genomic human DNA and unmethylated genomic human DNA was tested. In each case, 20 ng DNA or 100 ng DNA per assay were used. The dilution series were tested in triplicate or in 9-fold determination.

Analytical Sensitivity – Part 2

Proportion of methylated DNA	Total DNA	ScreenYu Gyn® positive
10 %	20 ng	3 / 3
1 %	20 ng	9 / 9
0.1 %	20 ng	9 / 9
0.01 %	20 ng	4 / 9
0 %	20 ng	0 / 9
10 %	100 ng	3 / 3
1 %	100 ng	9 / 9
0.1 %	100 ng	9 / 9
0.01 %	100 ng	1 / 9
0 %	100 ng	0 / 9

The detection limit for a positive result of the marker ZNF671 is 0.1 % methylated DNA for a sample containing a total of 20 ng DNA or 100 ng DNA per assay.

12.1.2 Analytical specificity – detection of unmethylated DNA

The analytical specificity of the PCR assay was determined using unmethylated PCR fragments of 10 – 12 kb representing the human genome. A 5-fold determination was performed. Results are shown in the table below. The samples were classified as valid via the ACTB marker. Up to a concentration of 1,000 ng of unmethylated, bisulfite-converted DNA (biDNA), no false-positive ScreenYu Gyn® result was obtained.

Analytical specificity of the PCR assay

DNA used	ZNF671 Cq ≤ 42	ACTB Cq ≤ 42
100 ng unmethylated biDNA	0 / 5	5 / 5
250 ng unmethylated biDNA	0 / 5	5 / 5
500 ng unmethylated biDNA	0 / 5	5 / 5
1,000 ng unmethylated biDNA	0 / 5	5 / 5
1,000 ng genomic DNA	0 / 5	0 / 5

12.2 Precision

12.2.1 Repeatability

Two bisulfite-converted patient samples were tested in ten independent runs with the ScreenYu Gyn® assay (4 replicates each). In all 40 determinations, the PAP I sample had a negative ScreenYu Gyn® result and the CIN3 had a positive ScreenYu Gyn® result. Thus, the samples show 100 % repeatability.

12.2.2 Reproducibility

At five centres, 20 patient samples were tested with ScreenYu Gyn®. Each time, a new sample preparation and bisulfite treatment was performed by different people, using different qPCR devices (cobas z 480 Analyzer, LightCycler® 480 I, CFX96 Touch Real-Time PCR Detection System).
18 out of 20 patient samples were consistent. This yields a reproducibility of 90 %.

12.3 Accuracy

The accuracy of the ScreenYu Gyn® assay was verified via Sanger sequencing using 15 patient samples. Negatively tested patient samples showed no methylated cytosines in the respective genomic region. Positively tested patient samples showed the correct ZNF671 genomic region with a high degree of methylation within the sequence.

12.4 Precision

The precision as the sum of precision and correctness of the ScreenYu Gyn® assay is given.

12.5 Robustness

No interference was observed in smear samples spiked with SiHa cells when increased concentrations of the following substances were added to the sample:

- up to 0.5 % Lugol's solution
- up to 0.5 % acetic acid

12.6 Cut-off

The optimal assay cut-off was determined using the so-called Youden's Index. The following criteria must be met for a positive ScreenYu Gyn® result:

CFX96 Touch Real-Time PCR Detection System

$\Delta Cq_{ZNF671\text{-}ACTB} \leq 10$ → ScreenYu Gyn® result is positive

$\Delta Cq_{ZNF671\text{-}ACTB} > 10$ → ScreenYu Gyn® result is negative

Cobas z 480 Analyzer

$\Delta Cp_{ZNF671\text{-}ACTB} \leq 9$ → ScreenYu Gyn® result is positive

$\Delta Cp_{ZNF671\text{-}ACTB} > 9$ → ScreenYu Gyn® result is negative

12.7 Clinical performance evaluation

The patient samples used here were obtained from European clinics (Germany, Portugal).

Bisulfite treatment of patient samples with the EZ DNA Methylation-Lightning Kit was performed with a centrifugation speed of 18,000 xg and a desulphonation time of 20 minutes.

For the clinical performance evaluation of ScreenYu Gyn®, 616 patient samples were examined with the following prevalence distribution of findings: Pap I (n = 380; 61.7 %), CIN 1 (n = 47; 7.6 %), CIN 2 (n = 50; 8.1 %), CIN 3 (n = 132; 21.4 %), cervical cancer (n = 7; 1.1 %).

Based on the established cut-off, clinical sensitivity and specificity were calculated.

Clinical performance evaluation of ScreenYu Gyn®

Findings according to cytology / histology	Detection	CI 95 %
Pap I (n = 380)	9.33 %	6.6 % - 12.7 %
CIN 1 (n = 47)	23.91 %	12.6 % - 38.8 %
CIN 2 (n = 50)	35.42 %	22.2 % - 50.5 %
CIN 3 (n = 132)	62.88 %	54.0 % - 71.1 %
CxCa (n = 7)	100.00 %	59.0 % - 100 %

Clinical performance data CIN 3+ / Pap I	Value	CI 95 %
Sensitivity	64.75 %	56.2 % - 72.7 %
Specificity	90.67 %	87.3 % - 93.4 %
Positive predictive value	72.00 %	63.3 % - 79.7 %
Negative predictive value	87.40 %	83.7 % - 90.5 %
Positive Likelihood Ratio	6.96	-
Negative likelihood ratio	0.39	-

CI = confidence interval

13 LIMITS OF THE PROCEDURE

- The interpretation of the ScreenYu Gyn® results should always be carried out in conjunction with results of further laboratory diagnostic procedures, as well as taking into account the clinical picture.
- The specifications according to the instructions for use, e.g., pipetting volumes, incubation times, temperatures and preparation steps must be adhered to in order to avoid erroneous results.
- Proper sampling and storage are critical to test results.
- In principle, it cannot be excluded in molecular biological test procedures that further very rare sequence variants could influence the test result, which are not yet covered in the sources consulted for the specificity and sensitivity analysis of the primers and probes.
- Non-specification instrument performance, as well as deviations from the described test procedure, specified storage conditions, materials, equipment, or recommended sample material, may result in differences from results obtained when all specifications are met.
- The provided internal and external controls are aids for the detection of faults. However, they cannot detect every possible fault. It is the user's responsibility to validate any modifications made or, if necessary, the devices used and to ensure compliance with the device specifications.

14 REFERENCES

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[7] International Agency for Research on Cancer (2008). European guidelines for quality assurance in cervical cancer screening – Second edition

15 LIABILITY

The ScreenYu Gyn® Kit may only be used in accordance with its intended purpose. Oncgnostics GmbH assumes no liability for any other use (e.g., non-compliance with these operating instructions and improper use) and any resulting damage.

16 QUESTIONS AND PROBLEMS

If you have any questions about or problems with the product, please contact your oncgnostics GmbH representative.

You can reach oncgnostics GmbH's technical support from Monday to Friday between 8 a.m. and 4 p.m. under the following phone number: +49 (0) 3641 5548500

Outside of office hours, e-mail us at: screenyugyn@oncgnostics.com

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17 ADDITIONAL NOTES

- Regulatory notice to customers in the European Union: Please note your obligation to report to your competent authority and to oncgnostics GmbH any serious incidents that have occurred in connection with the product.
- The current version of the safety data sheet for this product is provided in the Download Centre on the website (http://www.oncgnostics.com/en/downloadcenter/) or can be requested by e-mail to **screenyugyn@oncgnostics.com**.

18 MEANING OF THE SYMBOLS

Symbol	Meaning	Symbol	Meaning
PCR-MM	Mastermix		Storage temperature
STRIPS	PCR strips		If unopened, usable until (YYYY-MM-DD)
CAPS	Caps		Content sufficient for <n> tests
CONTROL +	Positive control		Manufacturer
H_2O	Water		Observe instructions for use
IVD	in vitro diagnostic		Protect from sunlight
LOT	Lot designation		Do not reuse
REF	Reference number	CE	CE marking

19 LIST OF CHANGES

Previous version (Release date)	Changes
5 (2024-11)	- Correction of the date format of the revision according to ISO 20417 on the cover page and in chapter 19. - Chapter 11.4 Bisulfite treatment of samples • Reformatting the workflow steps that need to be processed in addition to the manufacturer's instructions from Zymo Research. • Adding a warning notice stating that dry centrifugation is mandatory. - Chapter 11.5.1 Preparation and pipetting of the PCR • Adding a note regarding the storage of the remaining eluate after pipetting the PCR. • Clarification that Strips are to be sealed with unused Caps - Adaption of chapter 20 according to changes in chapter 11.4 and 11.5.1.

20 SHORT PROTOCOL

Below you will find a template of a quick guide in the form of a checklist.
Before using the Quick Start Guide, read thoroughly the instructions for use described in detail in Chapter 11, including all notes.
The bisulfite kit is not included in the ScreenYu Gyn® Kit. Bisulfite treatment of samples must be performed using the EZ DNA Methylation-Lightning Kit (CE-IVD) (see Chapter 7 for reference information).

Samples preparation

- ☐ Vortex patient samples for 5 seconds at maximum speed and transfer 1 ml to 1.5 ml reaction tube
- ☐ Centrifuge samples for 5 minutes at 10,000 xg
- ☐ Remove and discard 900 µl of supernatant above the pellet

Bisulfite treatment of samples

- ☐ Prepare EZ DNA Methylation-Lightning Kit (Zymo Research Europe GmbH) according to the manufacturer's instructions
- ☐ Resuspend pellet
- ☐ Setup reaction in a 0.5 ml reaction tube, vortex and centrifuge

Reaction for the bisulfite conversion

Component	Per reaction
Lightning Conversion Reagent	130 µl
Resuspended sample	20 µl
Total volume	150 µl

- ☐ Carry out the bisulfite treatment according to the manufacturer's instructions for the EZ DNA Methylation-Lightning Kit and follow the additional steps:
- ☐ Discard the flow through after the last wash step
- ☐ Centrifuge column in the empty Collection Tube for 1 minute at full speed to dry it completely
- ☐ Transfer column to 1.5 ml reaction tube
- ☐ Pipette 15 µl of M-Elution Buffer onto column and centrifuge at 8,000 xg for 30 seconds

Preparation and pipetting of the PCR

- ☐ Centrifuge sample
- ☐ Vortex and centrifuge ScreenYu Gyn® Mastermix
- ☐ Remove the PCR caps from the ScreenYu Gyn® Strips and discard them
- ☐ Pipette 10 µl ScreenYu Gyn® Mastermix per well
- ☐ Pipette 10 µl sample or ScreenYu Gyn® Positive Control (in well A1) or ScreenYu Gyn® Water (in well B1)
- ☐ Close ScreenYu Gyn® Strips with unused ScreenYu Gyn® Caps (transparent bag)
- ☐ Vortex and centrifuge ScreenYu Gyn® Strips

Performing the PCR

- ☐ Switch on the Real-time PCR device, open software if necessary and select ScreenYu Gyn Template
- ☐ Name PCR run individually, edit plate layout, check temperature protocol

PCR temperature protocol

Programme Name	Number of cycles	Temperature	Time (m:ss)
Initialization	1 x	94 °C	1:00
Amplification	42 x	94 °C	0:15
		61 °C	0:30
Cooling	1 x	37 °C (cobas z 480 Analyzer)	1:00
		30 °C (CFX96 Real-Time PCR Detection System)	

- ☐ Place ScreenYu Gyn® Strips and Balance Strips into the device and start PCR run

Analysis and interpretation of PCR data

- ☐ Open the exported file and merge data in a suitable spreadsheet programme
- ☐ Format the data so that the results of the different samples are displayed one below the other and the ZNF671 and ACTB markers are displayed side by side
- ☐ Check the results of the positive control and negative control for both markers
- ☐ Analyse the result for the collected samples

A sample is considered positive in the ScreenYu Gyn® assay when the following criteria are met:

Validity and positivity criteria

PCR device	Marker	Ct value	$\Delta Ct_{ZNF671 - ACTB}$
cobas z 480 Analyzer	ACTB	≥ 20, ≤ 32	-
	ZNF671	≥ 20, ≤ 42	≤ 9.00
CFX96 Real-Time PCR Detection System	ACTB	≥ 20, ≤ 32	-
	ZNF671	≥ 20, ≤ 42	≤ 10.00

A positive ScreenYu Gyn® result is associated with the presence of cervical intraepithelial neoplasia or cervical cancer. ScreenYu Gyn® should not be considered as the final therapeutic decision and must be analysed in conjunction with other medical findings.