WID®-easy PCR Kit

Realtime PCR Amplification Kit

Instructions for Use (IFU)

RUO For research use only.

WIDHB01v4en
12 February 2026

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LOT Batch code

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Notice of Change

Please note the following adaptions compared to the previous handbook version:

Document code	Changes	Date
WIDHB01v1en	Initial release of the handbook	17.11.2023
WIDHB01v2en	▪ Editorial changes ▪ Reference to the need to calibrate the PCR plates in chapter "General laboratory equipment" ▪ Changes in recommended baseline settings, cutoff values and Cq-range for COL2A1 in chapter “Data Analysis” ▪ Changes in device-specific adjustments in table 8. ▪ Advice and reference to expected Cq-values of gBlock samples in table 9. ▪ Reference to the need to validate cq cutoff levels in the chapter "Sample analysis"	19.08.2024
WIDHB01v3en	▪ Addition of an Intended Use (page 5) ▪ Update to Reagent storage and handling (page 7) ▪ Update to device-specific adjustments in table 8. ▪ Update to expected Cq-values of gBlock samples in table 9. ▪ Update to Sample Analysis (page 21) and addition of Table 10	03.04.2025

	example PCR results and interpretation	
WIDHB01v4en	▪ Update to Intended Use (page 5) ▪ Update to Product Description (page 5) ▪ Update to Summary and Explanation (page 5) ▪ Update to General laboratory equipment (page 7 and 8) ▪ Update to Specimens and test samples (page 9) ▪ Update to Table 1 expected Cq-ranges for controls (page 21) ▪ Update to contact information (page 22 and 28) ▪ Update to PMR calculation (page 24) ▪ Update to chapter name Analytical Performance Characteristics (page 25)	12.02.2026

For any further questions, please contact us at:

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Content

Intended Use

The WID®-easy PCR Kit consists of PCR reagents used to determine the qualitative methylation state of *GYPC* and *ZSCAN12* gene regions in human genomic bisulfite-converted DNA from cervicovaginal specimens.

Product Description

The WID®-easy PCR Kit is a relative-quantitative PCR-based multiplex assay for the detection of DNA methylation in *GYPC* and *ZSCAN12* gene regions as a Percentage of Methylated Reference (PMR) value via qPCR measurement of Cq-values. Human genomic, bisulfite-converted DNA from cervicovaginal specimens is used for analysis.

The assay must be used only by qualified and trained personnel in a professional laboratory environment. The optimal input amount under standard conditions is 20 ng bisulfite-converted DNA per PCR reaction.

Summary and Explanation

DNA methylation is a modification of the DNA which is essential in the epigenetic regulation of gene expression. It involves the addition of a methyl group to the 5th position of the pyrimidine ring of the cytosine nucleotide in the regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide (CpG) in the linear sequence of bases along its 5' → 3' direction. These CpG sites occur with high frequency in genomic regions called CpG islands, which are frequently located in the promoter region of genes. During carcinogenesis, several of these CpG islands become methylated [1-3] including CpG islands of the *ZSCAN12* and *GYPC* genes in endometrial and cervical cancer [4-9]. The WID®-easy PCR assay enables the detection of methylation of the genes *ZSCAN12* and *GYPC* on human genomic bisulfite-converted DNA from cervicovaginal specimens using *COL2A1* as an internal control for DNA input and efficacy of bisulfite conversion.

Table 2 List of detected methylation sites

Assay	Gene	Methylation site
Primer Mix A	*ZSCAN12*	Chr6: 28367535-28367599
Primer Mix B	*GYPC*	Chr2: 127413797-127413902
Primer Mix B	*COL2A1*	Chr12: 48381229-48381320

Materials provided

Table 3 Kit content

Reagent	Cap Color		Volume per kit (100 reactions, 2x 50)	Storage
Nuclease-Free Water	Blue		1 x 1.5 mL	-25 °C to -15 °C, protected from light
Master Mix	Black		1 x 1 mL	
Positive Control	White		1 x 60 µL	
Primer Mix A	Red		1 x 50 µL	
Primer Mix B	Yellow		1 x 50 µL	
Standard 1	Green		1 x 60 µL	
Standard 2	Green		1 x 60 µL	
Standard 3	Green		1 x 60 µL	
Standard 4	Green		1 x 60 µL	

NOTE

Please note that the packaging size describes the number of PCR reactions **without** taking into account the number of required controls or the required excess for pipetting.

NOTE

The kit contains reagents to perform up to 50 reactions with each Primer Mix.

Reagent storage and handling

The WID®-easy PCR Kit is shipped on dry ice. The kit´s components should arrive frozen, except the Master Mix, which is stored in a buffer that prevents freezing of the reagent.

Please check the completeness of the kit upon receipt. If one or more components are not frozen or if tubes or packaging have been compromised during shipment, please immediately contact Sola Diagnostics GmbH.

Store all components at -25 °C to -15 °C without light exposure and avoid thawing and freezing more than 5 times. Primer Mix A and Primer Mix B must be protected from light.

The Positive Control and the Standards should be stored separately from PCR reagents.

The expiry date of the kit is indicated on the kit box label.

Material and devices required but not provided

General laboratory equipment

- Desktop centrifuge with a rotor for 2 mL reaction tubes
- Centrifuge with a rotor for microtiter plates
- Vortex mixer
- Calibrated adjustable pipettes with disposal aerosol tight filter tips
- Calibrated* 200 µL 96-well or 384-well reaction plates with calibrated optical adhesive foil; PCR grade. PCR plates and foils need to be calibrated on your instrument!
- Suitable racks and cooling racks for 2 mL tubes
- Disposable powder-free gloves

- Qubit Fluorometer (cat. no. Q33238, Thermo Fisher Scientific) or similar.
- PCR Workstation or Clean Bench
- qPCR Thermocycler with 96- or 384-well block and filters for FAM and CY5 (potentially ROX as background filter)

* Depending on the device manufacturer

NOTE

All materials to be used for PCR shall be of appropriate quality (DNA-free and for molecular biology). Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer's instructions and recommendations. Only use calibrated PCR plates and foils.

Reagents, kits, and consumables

Table 4 Reagents required but not provided

Reagent	Supplier	Order number
Standard genomic DNA extraction kit	NA	NA
EZ DNA Methylation-Lightning Kit	Zymo Research	D5030-E
Qubit™ dsDNA BR Assay QuantiFluor ONE dsDNA System	Thermo Fisher Scientific Promega	Q32850 E4871
TE Buffer pH 8.0 (Ambion) or Nuclease-Free Water	Fisher Scientific GmbH Thermo Fisher Scientific	10742317/ AM9932

Instruments and software

The WID®-easy PCR Kit was verified to be used with the following PCR instrument:

- QuantStudio™ 5 and 7 Real-Time PCR System (cat. no.: A28139 and A43185 Thermo Fisher Scientific) with QuantStudio™ Design and Analysis Software (v. 1.5 and above)

Technical transfer data are available for:

- LightCycler® 480 II, 96-well (05015278001, Roche Diagnostics (Schweiz) AG)

NOTE

Please ensure that all instruments used have been installed, calibrated, checked, and maintained according to the manufacturer's instructions and recommendations.

NOTE

The application of the WID®-easy PCR Kit on instruments other than the above-mentioned ones are at users risk and must be validated before use.

Specimens and test samples

The following specimen has been verified with the WID®-easy PCR Kit: Genomic DNA isolated from human cervicovaginal specimens after bisulfite conversion. Please refer to the limitations of use section.

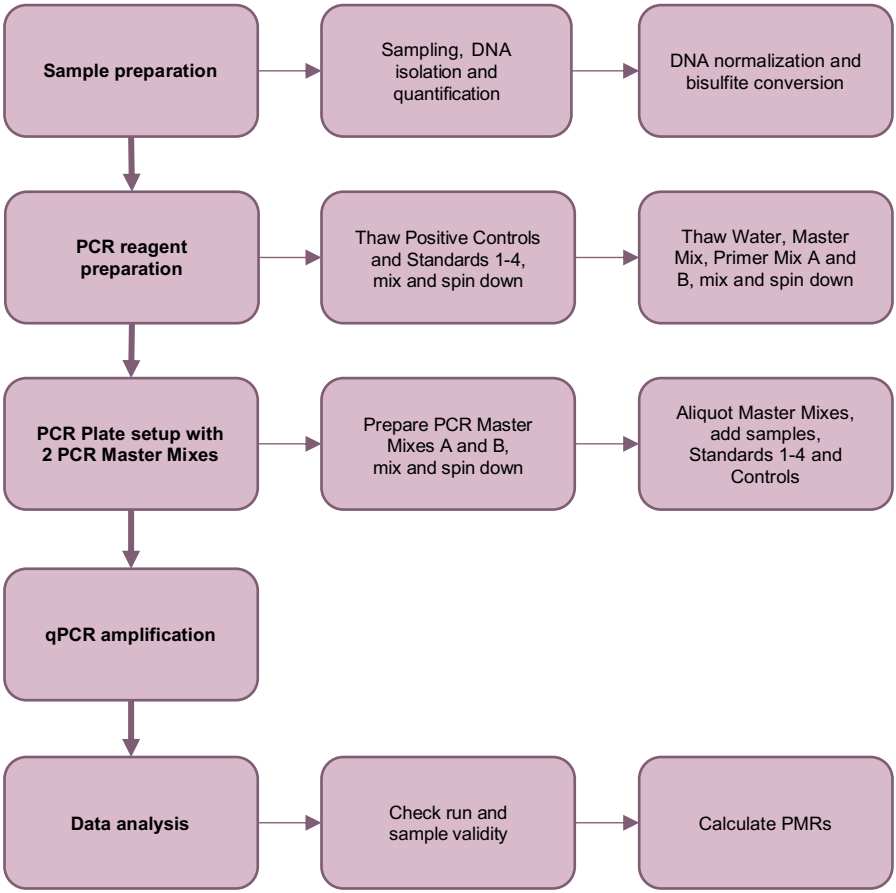
The obtained unmodified genomic DNA shall be stored undiluted at -25 °C to -15 °C.

Warnings and Precautions

- Read the instructions carefully before using the product.
- Read the Safety Data Sheets (SDS) for all products, which are available on request. Please contact the respective manufacturers for copies of the SDS for any additionally needed reagents.
- Kit components of different kit lots must not be mixed.
- Kit components shall not be aliquoted into secondary reaction vessels.
- The use of this product is limited to personnel specially instructed and trained in PCR techniques.
- Before the first use, check the product and its components for:
  - Integrity
  - Completeness with respect to number, type, and filling (see chapter Materials provided)
  - Correct labelling
  - Frozenness upon arrival.
- Do not use a kit that has passed its expiration date.
- Discard sample and assay waste according to your local safety regulations.
- All instruments used must be installed, calibrated, checked, and maintained according to the manufacturer´s instructions and recommendations.

Procedure

Overview of the experimental workflow



Sample preparation

DNA extraction

DNA extraction and purification should be carried out from human cervico-vaginal specimens according to the supplier's instructions using a standard genomic DNA isolation method, which can be bead- or column-based.

The DNA must be quantified directly after the extraction.

DNA quantification and dilution

Quantification of the DNA should be carried out by a fluorometric quantification method using the Qubit™ 3.0 Fluorometer or a similar fluorescent-based instrument. Use the Qubit™ dsDNA BR Assay or a similar product according to the manufacturer's protocol. Dilute the DNA to a final concentration of 10 ng/µL. Using a DNA concentration lower than 10 ng/µL is not recommended for further processing. Repeat DNA extraction or sampling if needed.

NOTE

For sample dilution we recommend the use of 1x TE (Tris-EDTA)-Buffer, pH value 8.0 or nuclease-free H_2O and a sample volume > 1.5 µL.

DNA storage

Store the unmodified DNA samples at -25 °C to -15 °C. Undiluted DNA samples can be stored for 4 weeks at 2 °C to 8 °C or at -25 °C to -15 °C for long-term storage.

Bisulfite conversion

Bisulfite conversion should be carried out using the Zymo Research EZ DNA Methylation-Lightning Kit (bead- or column-based) according to the manufacturer's instructions using **200 ng DNA (equals 20 µL of 10 ng/µL diluted DNA)**. Elute with 50 µL Nuclease-Free Water to reach a final concentration of 4 ng/µL (corresponds to 20 ng within 5 µL volume per PCR

reaction). Using a bisulfite-converted DNA amount below 20 ng per PCR reaction will result in low PCR yields, and the signal might fall below the target-specific detection limits, which can lead to inconclusive PCR results.

NOTE

Wash buffer used during bisulfite conversion may have potential interference on amplification performance. Therefore, wash steps within the bisulfite conversion assay must be followed.

NOTE

Bisulfite-converted DNA is not suitable for long-term storage and should be used immediately after conversion. Storage of unmodified genomic DNA or the sample material is recommended instead. If immediate processing is not possible, bisulfite-converted DNA can be stored for up to 7 days at -80°C.

Standard 1-4 and Positive Control preparation

Thaw the Standard 1-4 (green cap) and the Positive Control (PC; white cap), and homogenize it by gentle vortexing followed by brief centrifugation.

Provided standards (Standard 1-4) and Positive Control (PC) must be assessed in duplicates per each PCR run using 5 µL of reagent per well and replicate. No additional dilution steps are required.

PCR Master Mix setup

Remove and thaw the following components from the WID®-easy PCR Kit:

- Nuclease-free water (blue cap)
- Primer Mix A (red cap)
- Primer Mix B (yellow cap)

- Master Mix (black cap)

During the PCR Master Mix setup, it is recommended to keep the Master Mix in a cooled environment (e. g., on a cooling rack). All frozen components need to be thawed at room temperature (15 °C to 30 °C, ca. 20 min, protected from light) and homogenized by inverting the tubes (Master Mix) or gently vortexing (Primer Mix). The reagents should be then briefly centrifuged (approx. 10 s).

NOTE

Make sure to analyze each sample with each Primer Mix to obtain the complete information of the assay. Therefore, prepare two separate PCR Master Mixes, one for each Primer Mix. It is recommended to analyse samples in duplicates with both Primer Mixes.

Prepare a PCR Master Mix for each Primer Mix (2 separate PCR Master Mixes for each sample) according to Table 5 (next page) for the total number of samples to be tested in an appropriately sized microcentrifuge tube in a dedicated clean area. Include at least two reactions for the PC, the No Template Control (NTC; water as sample), as well as each Standard 1-4 (STD 1-4) for each PCR Master Mix (A & B) into your calculation. Samples, STD 1-4, NTC and PC, must be analyzed in duplicates.

Example of a plate layout for 18 samples:

	PCR Mix A					PCR Mix B					
	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	S9	S13	S17	STD1	S1	S5	S9	S13	S17	STD1
B	S1	S5	S9	S13	S17	STD1	S1	S5	S9	S13	S17	STD1
C	S2	S6	S10	S14	S18	STD2	S2	S6	S10	S14	S18	STD2
D	S2	S6	S10	S14	S18	STD2	S2	S6	S10	S14	S18	STD2
E	S3	S7	S11	S15	NTC	STD3	S3	S7	S11	S15	NTC	STD3
F	S3	S7	S11	S15	NTC	STD3	S3	S7	S11	S15	NTC	STD3
G	S4	S8	S12	S16	PC	STD4	S4	S8	S12	S16	PC	STD4
H	S4	S8	S12	S16	PC	STD4	S4	S8	S12	S16	PC	STD4

NOTE

It is recommended to divide the plate evenly with the same layout for PCR Master Mix A and PCR Master Mix B to simplify the workflow. During run setup, assign all tested targets to corresponding wells.

NOTE

If you are testing fewer than 10 reactions, use enough PCR Master Mix for one extra sample. If you are testing 10 or more reactions, use an excess reagent PCR Master Mix volume of +10%.

Table 5 PCR Master Mix reaction setup

Component	Volume		
	# 1 rxn	# 10 rxn	# 50 rxn
Nuclease-Free Water	4 µL	40 µL	200µL
Primer Mix A **OR** Primer Mix B	1 µL	10 µL	50 µL
Master Mix	10 µL	100 µL	500 µL
Total volume	**20 µL**	**150 µL**	**750 µL**
Bisulfite-converted DNA template or Positive Control or Standard or No Template Control	5 µL	10 x 5 µL	50 x 5 µL

Gently vortex the PCR Master Mix without generating bubbles, followed by brief centrifugation.

Application of PCR Master Mixes and DNA templates and controls

Aliquot 15 µL of the PCR Master Mix per well in an appropriate 96-well or 384-well PCR plate.

Add 5 µL of the following sample types to the prepared PCR plate containing the PCR Master Mixes:

NTC: add 5 µL of Nuclease-Free Water instead of a sample.

DNA Sample: add 5 µL of the prepared, diluted bisulfite-converted DNA samples (4 ng/µL). Technical replicates are required for quantitative analysis.

PC: add 5 µL of Positive Control instead of a sample.

Standard 1-4: add 5 µL of Standard 1-4 instead of a sample.

NOTE

Use at least two technical replicates for each sample/component per PCR Master Mix (A & B). Otherwise, the run cannot be validated.

Close the PCR plate with an optical adhesive foil suitable and validated for your qPCR instrument, gently mix and spin down.

PCR amplification

Program the qPCR instrument with the following amplification program. Make sure to set the ramping to 1.6 °C/s. Perform a hot-start PCR in order to activate the polymerase and prevent the formation of non-specific amplification products.

Table 6 PCR protocol

Temperature	Time	Cycles
95 °C	1 min (hot-start for activation of the polymerase)	1 x
95 °C	15 s	45 x
60 °C	30 s	

NOTE

If qPCR instruments with rapid heating and cooling steps (> 2 °C/s) are used, **ramping is required to be adjusted to 1.6 °C/s** to provide an optimal kit performance.

Define the following properties for the correct signal detection during the plate setup:

Table 7 Properties for the correct signal detection during the qPCR run

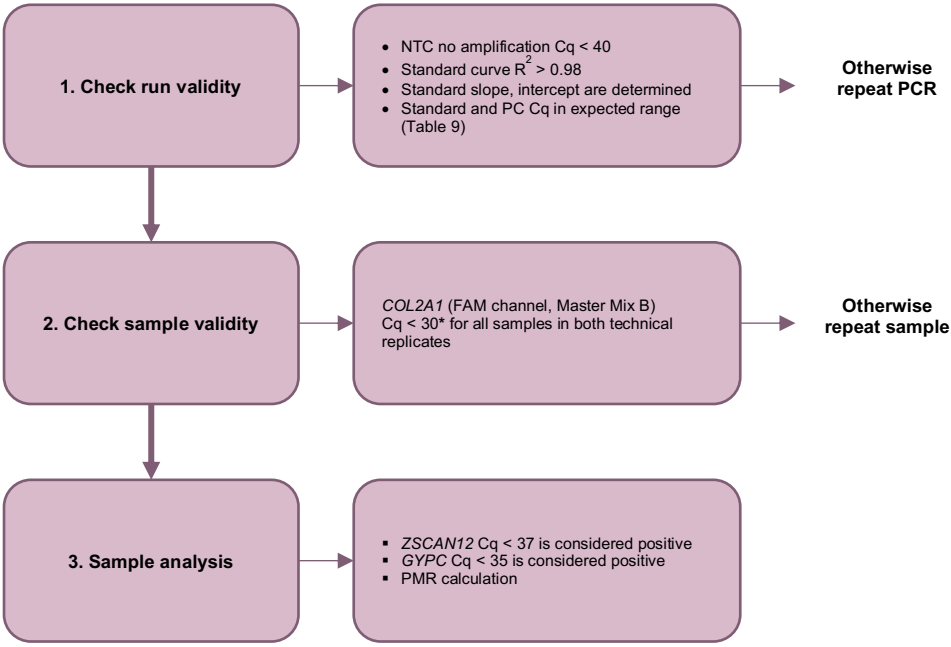
Primer Mix	Target	Reporter (Fluorescence)	Quencher
A	*ZSCAN12*	FAM	Dark quencher
B	*GYPC*	Cy5	Dark quencher
B	*COL2A1*	FAM	Dark quencher

NOTE

For basic information regarding the setup, programming, and maintenance of the different qPCR instruments, please refer to the user manual of the respective instrument.

Data Analysis

Workflow overview



* The *COL2A1* cutoff value for determining a positive sample depends on device-specific parameters for baseline and threshold and may be adjusted according to your setup. Ideally, samples should be in a Cq range between Cq > 25 and Cq < 30 if 20 ng input material is used. Contact customer support in case of questions.

General settings

- Use the standard curve method, for instruments that require a passive reference dye, use ROX.

Table 8 Standards used in the data analysis

Sample	Quantity [copies per reactionl
Standard 1	100,000
Standard 2	10,000
Standard 3	1,000
Standard 4	100

Table 9 Specific thresholds used in the data analysis

Primer Mix	Target	Reporter (Fluorescence)	Threshold in ΔRn	Baseline
A	*ZSCAN12*	FAM	0.22*	1-16*
B	*GYPC*	Cy5	0.04*	1-18*
B	*COL2A1*	FAM	0.1*	6-20*

* These thresholds and baselines were used during assay verification with the above-mentioned instrument and software version and need to be adjusted for different instruments and softwares. Device-specific adjustments need to be done during tech transfer; see troubleshooting. Baseline and Threshold should be adjusted in a way that Cq values for Standards and PC are in the expected Cq ranges (Table 9).

NOTE

To set up an experiment in the QuantStudio™ Design and Analysis Software, create a template file with the desired samples and the above-mentioned parameters. This can be saved and re-used to standardize analysis settings.

Run validation

- Check if **NTC** shows **no amplification**
- Check if **PC** and **Standard 1-4** show amplification of all targets with Cq-values in the expected ranges

Table 10 expected Cq-ranges for controls*

Sample	*ZSCAN12*	*GYPC*	*COL2A1*
PC	26 – 28.5	23 - 26	26 - 29
Standard 1	20 - 23	18 - 21	21 - 23
Standard 2	23 – 26.5	21 - 24	25 - 27
Standard 3	26 – 29.5	25 – 27.5	28 - 31
Standard 4	29.5 - 33	28 - 31	31 - 35
Slope	-3.7 - -3.1	-3.6 - -3.1	-4.2 - -3.1

* All Cq values depend on device-specific parameters for baseline and threshold. Both may slightly differ in your setup, but ideally, Cq values should be in the expected range after threshold and baseline adjustment. Contact customer support in case of questions.

NOTE

Compliance with above mentioned Cq and slope ranges for Standards and PC is important for correct sample analysis.

Sample analysis

View the amplification plots for the entire qPCR run. A detailed analysis of raw data depends on the qPCR instrument used.

The "baseline noise levels" should either be automatically defined or predefined for specific cycles (e. g., 3 to 15). Use the NTC to determine the respective baseline. Set the thresholds for all targets according to your previously defined settings (see Run validation).

Verify that Standard and PC mean Cqs and slopes are within the above-mentioned ranges (Table 9). If Standards, PC, NTC and slope are valid (see Run validation), continue with the sample data analysis.

Information for data export and data processing can be found in the respective manuals of the real-time instrument manufacturer. Export the raw data (information should at least include the "sample name", “Cq-values” and “quantity”, as well as the respective means (optional) for subsequent calculations).

NOTE

If other qPCR instruments than the QuantStudio™ 5 are used, the data analysis steps need to be adjusted, and the procedure needs to be verified in the user’s responsibility.
Contact the Customer Support Team if you need further information.

Data analysis/verification

For a valid sample, the *COL2A1* in the FAM channel should have a Cq-value below 30 in both duplicates to be measured within the range of highest assay sensitivity and specificity. If this is not the case, the sample must be repeated with re-quantified and adjusted bisulfite-converted DNA.

In a valid sample, *ZSCAN12* with a Cq-value below the cutoff of Cq < 37 and/or *GYPC* with a Cq-Value below the cutoff of Cq < 35 is considered a positive PCR amplification. If the Cq value is above the Cq cutoff, it is deemed as no amplification and set to ‘undetermined’

Both technical replicates need to have a target amplification below or above the above mentioned/pre-defined Cq cutoffs. If a sample shows an inconclusive result (one technical replicate < Cq cutoff, the 2nd technical replicate > Cq cutoff) for one of the two targets, the qPCR needs to be repeated with a higher input amount of BC-DNA (40 ng).

Cq cutoff levels for *COL2A1*, *ZSCAN12* and *GYPC* can be adjusted during tech transfer but must be validated. Contact customer support in case of questions.

Table 10 example PCR results and interpretation

		COL2A1 Cq1	COL2A1 Cq2	GYPC Cq1	GYPC Cq2	ZSCAN12 Cq1	ZSCAN12 Cq2
before cutoff was applied	**Sample 1**	30.2	30.9	33.2	32.9	35.2	35.5
after cutoff was applied		Undet.	Undet.	33.2	32.9	35.2	35.5
conclusion	**Sample needs to be repeated due to undetermined (>cutoff) COL2A1 value.**						
before cutoff was applied	**Sample 2**	28.1	27.8	35.2	34.7	36.9	36.5
after cutoff was applied		28.1	27.8	Undet.	34.7	36.9	36.5
conclusion	**Sample 2 needs to be repeated due to undetermined (>cutoff) GYPC Cq value.**						
before cutoff was applied	**Sample 3**	28.1	27.8	33.9	34.7	37.1	36.5
after cutoff was applied		28.1	27.8	33.9	34.7	Undet.	36.5
conclusion	**Sample 3 needs to be repeated due to undetermined (>cutoff) ZSCAN12 Cq value.**						
before cutoff was applied	**Sample 4**	28.1	27.8	36.1	35.9	37.1	37.3
after cutoff was applied		28.1	27.8	Undet.	Undet.	Undet.	Undet.
conclusion	**No PCR amplification in both targets → ∑PMR=0**						
before cutoff was applied	**Sample 5**	28.1	27.8	33.2	32.9	35.2	35.5
after cutoff was applied		28.1	27.8	33.2	32.9	35.2	35.5
conclusion	**Positive PCR amplification in both targets → ∑PMR calculation**						

PMR calculation

Most qPCR analysis software tools allow the automatic calculation of the target quantities when the standard curve is properly set up. If this should not be the case in your software, you should manually calculate the quantity Q for all samples and both Positive Control replicates with the following formula (1).

$$Q = 10^{\frac{Cq(target) - Intercept(target\ Std\ curve)}{Slope(target\ Std\ curve)}} \quad (1)$$

Next, calculate the Percentage of Methylated Reference (PMR) per sample with formula (2) and (3), where $Q_{S\ ZSCAN12}$, $Q_{S\ GYPC}$ and $Q_{S\ COL2A1}$ refer to mean quantity values of one sample, while $Q_{PC\ ZSCAN12}$, $Q_{PC\ GYPC}$ and $Q_{PC\ COL2A1}$ refer to the mean quantity values obtained from the Positive Control.

$$PMR_{ZSCAN12} = \frac{\frac{\overline{Q}_{S\ ZSCAN12}}{\overline{Q}_{S\ COL2A1}}}{\frac{\overline{Q}_{PC\ ZSCAN12}}{\overline{Q}_{PC\ COL2A1}}} * 100 \quad (2)$$

$$PMR_{GYPC} = \frac{\frac{\overline{Q}_{S\ GYPC}}{\overline{Q}_{S\ COL2A1}}}{\frac{\overline{Q}_{PC\ GYPC}}{\overline{Q}_{PC\ COL2A1}}} * 100 \quad (3)$$

Finally, calculate the sum of both PMR values according to formula (4).

$$\sum PMR = PMR_{ZSCAN12} + PMR_{GYPC} \quad (4)$$

For ΣPMR interpretation, please refer to Evans et al. [6]

Analytical Performance Characteristics

Analytical specificity

Non-bisulfite-converted human DNA was tested with the assay. There is no amplification on human DNA for *ZSCAN12* and *GYPC*.

Analytical sensitivity

The analytical sensitivity of the WID®-easy PCR Kit is defined as the concentration (copies/µL of the eluate) of bisulfite-converted DNA molecules of the target regions of *ZSCAN12* or *GYPC* that can be detected with a positivity rate of 95 %. The analytical sensitivity was confirmed by analysis of dilution series of artificial DNA of the methylated targets *ZSCAN12* and *GYPC*. The confirmation was carried out on five different days with 6 replicates per concentration.

For the detection of *ZSCAN12* and *GYPC*, the analytical sensitivity is < 1 copies/µL with a 95 % CI (Confidence Interval).

Precision

The precision of the WID®-easy PCR Kit was determined as inter-assay variability (variability between different experimental setups) and inter-lot variability (variability between different production lots).

For the inter-assay variability, the study was run on three different setups (three instruments, three operators) on five days and in five replicates. For the analysis, the SD (Standard Deviation) and Coefficient of Variation (% CV) of repeatability, of reproducibility, and of within-laboratory precision were calculated as per the CLSI EP05 (4.6.2) guidelines. With regard to the Cq-value results, the % CV of repeatability, reproducibility, and within-laboratory precision did not exceed 5 %, indicating that the performance of the assay is not affected by the testing procedure.

For the inter-lot variability, two different production lots were tested at two different bisulfite converted DNA concentrations. The SD and coefficient of variation (% CV) were calculated as per the CLSI EP05 (4.6.2) guideline. The *ZSCAN12* and *GYPC* assays show a % CV < 5 % for all samples,

including positive control and standards. In conclusion, the assay performance is not affected by different kit lots.

Limit of blank

This study followed CLSI EP-17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.

The LOB (Limit of Blank) was performed using at least two kit lots with eight replicates measured on three different days; in total, 120 wells were measured.

The LOB for *ZSCAN12* is Cq 43.0 and for *GYPC* is Cq 42.4.

Linear range

The linear range of the WID®-easy PCR Kit was evaluated by analyzing a logarithmic dilution series of bisulfite converted DNA for both targets *GYPC* and *ZSCAN12* using concentrations ranging from 10 to 100,000 copies/reaction, and each dilution was analyzed in four replicates.

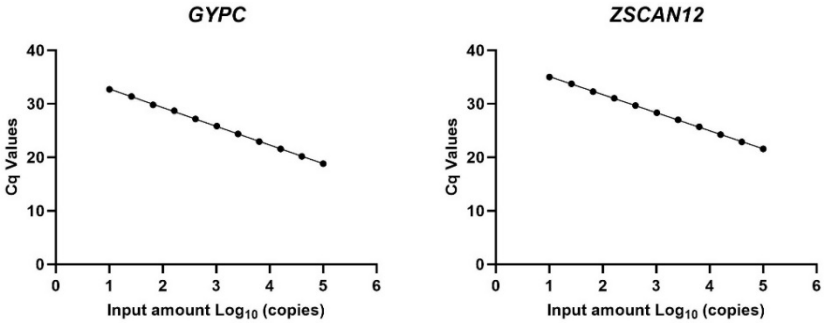


Figure 1: Linear regression of the dilution series plotted against the Log10 concentrations for the *ZSCAN12* (left) and *GYPC* (right).

The linear range was defined for concentrations from 10 to 100,000 copies for both assays. The linearity coefficient was calculated at R^2= 0.9998 for *ZSCAN12* and R^2=0.9991 for *GYPC*. The measured slopes were -3.376 for the *ZSCAN12* assay and -3.492 for the *GYPC* assay.

Troubleshooting

The troubleshooting guide may be helpful for solving any problems that may arise. Customer support is happy to answer any questions about the information and protocols given in this handbook (see Technical Assistance).

Failure	Comments and suggestions
Invalid No Template Control (NTC)	1. Targets and/or internal controls were detected in NTC. Contamination occurred during the preparation of the qPCR. Repeat the qPCR with new reagents in replicates. Where possible, close the tubes directly after adding reagents. Ensure that the workspace and the instruments are decontaminated at regular intervals. Change gloves occasionally. 2. Check the maintenance interval for all used devices (e.g., pipettes)
Invalid Positive Control (PC) or Standards	1. Incomplete detection of targets, and/or internal controls. Incorrect handling occurred during the preparation of the qPCR and/or positive control. Repeat the qPCR with new reagents in replicates. If possible, close the PCR tubes directly after adding the sample to be tested. Ensure that the workspace and the instruments are decontaminated at regular intervals. 2. Check the maintenance interval for all devices used (e.g., pipettes, PCR cycler). Make sure that all devices that are used are calibrated (including PCR plates). 3. Check the Master Mix calculation and preparation protocol for errors. High Cq-values indicate low Primer Mix and Master Mix concentrations in the reaction.
Cq-values of Positive Control / Standard are below the valid range	Check the Cq-threshold in the analysis view of your qPCR analysis software. It should be above the baseline, and the threshold must be set in the linear phase of the amplification plot. For target *GYPC,* it is of great importance to determine your qPCR instrument-specific threshold with a blank and a positive sample as low as possible but still safely above the background to ensure maximum assay sensitivity. Please contact the technical support if any assistance is required for this step.

Cq-values of Internal Control *COL2A1* are above the valid range (Cq > 30)	1. Repeat the DNA quantification input to ensure a minimum of 20 ng bisulfite converted DNA per reaction. Adjust the sample concentration if possible. 2. If the concentration in the gDNA (genomic DNA) sample is sufficiently high, repeat the analysis directly after a fresh bisulfite conversion step.
The storage conditions for one or more kit components do not comply with the instructions given in Reagent storage and handling	Check the storage conditions and the expiration date (see the kit box label). Use a new kit if the reagents were stored improperly.

Quality Control

All kit components undergo an intensive quality assurance process at Sola Diagnostics GmbH. The quality of the test kits is permanently monitored to ensure unrestricted usability. Please contact us if you have any questions regarding quality assurance.

Technical Assistance

For technical advice, please contact our Customer Support Team:

E-Mail: service@wid-easy.info

References

1. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer 2011;11(10):726-34. DOI: 10.1038/nrc3130.
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4. Barrett JE, Jones A, Evans I, et al. The WID-EC test for the detection and risk prediction of endometrial cancer. Int J Cancer 2023;152(9):1977-1988. (In eng). DOI: 10.1002/ijc.34406.
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Limitations of Use

- The procedures in this handbook must be followed as described. Any deviations may result in assay failure or cause erroneous results.
- Use of this product is limited to personnel specially instructed and trained in qPCR techniques.
- Appropriate specimen collection, transport, storage, and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The kit has only been verified using the kits described in chapter Reagents, kits, and consumables for DNA extraction and purification.
- The kit has only been verified for use with an optimum input of 20 ng human genomic bisulfite-converted DNA per reaction.
- Good laboratory practice is required to ensure the performance of the kit.
- Results must be interpreted by a trained professional user.
- Interpretation of results must account for the possibility of false negative and false positive results.
- Do not use expired or incorrectly stored components.

Ordering information

Direct your orders via email to order@wid-easy.info.

Product	Packaging size	Order number
WID®-easy PCR Kit	100 reactions (2x 50)	OE00083

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Explanation of Symbols

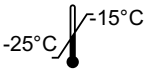
Manufacturer

Batch code

Contains sufficient reagents for <N> tests

Consult electronic instructions for use (eIFU)

Use-by date



Temperature limit

Catalogue number

Keep away from sunlight

Keep dry

Further marking used in this handbook:

Useful tips

Attention, be sure to follow this notice!

blue underlined text — Links leading to external content like homepages, e-mail addresses

black underlined text — Cross-links in the document for easy navigation

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