



PreCursor-M AnoGYN

Instructions For Use

For the assessment of hypermethylation of the genes

ZNF582 and *ASCL1*

FOR RESEARCH USE ONLY (RUO).

NOT FOR USE IN DIAGNOSTIC PROCEDURES.



Self-screen B.V., Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands



QHR16100



R_n

1

Contents

- Intended Use 3
- Summary and Explanation 3
- Materials Provided..... 3
- Materials required but not provided..... 4
- Reagent storage and handling..... 6
- Sample preparation and handling 6
- Procedure for the Rotor-Gene Q PCR machine..... 8
- Procedure for the Mic qPCR cycler..... 10
- Interpretation of results 12
- Validation criteria for successful analysis 13
- Calculating $\Delta\Delta C_t$ values 13
- Troubleshooting 14
- Limitations 15
- References 15
- Symbols 16
- Contact information..... 16
- Revision History 16

Intended Use

FOR RESEARCH USE ONLY (RUO). The result shall not be used for clinical diagnosis nor patient management. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

PreCursor-M AnoGYN is a multiplex real-time methylation-specific PCR assay for the detection of promoter hypermethylation of the human genes *ZNF582* and *ASCL1* in bisulfite-converted DNA prepared from human sample material.

This product is developed to be used on Rotor-Gene Q 5plex instruments from QIAGEN (5plex and 5plex HRM) or the Mic qPCR cycler from Bio Molecular Systems (Mic 4-Channel and Mic IVD).

Summary and Explanation

DNA methylation is a biochemical process that is important for normal development in higher organisms¹. It involves the addition of a methyl group to the 5th position of the pyrimidine ring of the cytosine nucleotide. Abnormal patterns of DNA methylation also play a major role in carcinogenesis and methylation patterns of certain genes have been identified in multiple cancer types, amongst which is anal and vulvar cancer²⁻⁷. PreCursor-M AnoGYN allows the detection of promoter hypermethylation of the genes *ZNF582* (*Zinc finger protein 582*) and *ASCL1* (*Achaete-Scute Family BHLH Transcription Factor 1*) on bisulfite-converted DNA prepared from human sample material and using β -*actin* as an internal sample quality control.

PreCursor-M AnoGYN uses primers and probes specific for methylated DNA of the two human target genes *ZNF582* and *ASCL1*. The sample reference β -*actin* (*ACTB*) is a methylation-unspecific PCR and is used for normalization and quality control. Amplification of the genes is detected with fluorescent hydrolysis probes. The multiplex format of the assay enables simultaneous amplification and detection of the three targets within one reaction. The C_T value (cycle threshold) represents the number of PCR cycles necessary for detection of a fluorescent signal above a background signal, which is correlated to the number of target molecules present in the sample. DNA methylation is quantified by $\Delta\Delta C_T$ values of the target genes *ZNF582* and *ASCL1*⁸. First the ΔC_T value is calculated by deducting the C_T value of the sample reference gene *ACTB* from the C_T value of the sample target gene. For normalization, the ΔC_T value of the calibrator is deducted from the ΔC_T of the sample target genes resulting in a $\Delta\Delta C_T$ value. The calibrator contains a standard concentration of low-copy DNA fragments of the three targets of the assay *ZNF582*, *ASCL1* and *ACTB*.

Materials Provided

	Volume per tube (at least)
PreCursor-M AnoGYN Master Mix	2 x 540 μ l
PreCursor-M AnoGYN Calibrator	2 x 50 μ l

Materials required but not provided

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

Consumables and reagents for bisulfite-conversion

Compatible bisulfite-conversion kits include:

- EZ DNA Methylation Kit™ (ZYMO Research, cat.no. D5001 or cat.no. D5002)
- EZ DNA Methylation Lightning™ (ZYMO Research, cat.no. D5030 or cat.no. D5031)

Consumables, reagents and equipment for real-time methylation-specific PCR

- Strip tubes and Caps, 0.1 ml for the Rotor-Gene Q (cat. no. 981103, QIAGEN) or strip tubes and caps, 0.1 ml for the Mic cycler (Bio Molecular Systems, cat.no. MIC-TUBES)
- Purified water (Molecular Biology Grade, distilled or deionized)

Equipment

- Adjustable pipets (1–10 µl; 10–100 µl) and filter tips
- Disposable gloves
- Benchtop centrifuge
- Vortex mixer
- Qubit® (Thermo Fisher Scientific, cat.no. Q33216), NanoDrop® 3300 Fluorospectrometer (Thermo Fisher Scientific, cat.no. ND-3300), or equivalent

Equipment for real-time PCR

- Real time PCR systems: the assay is developed for use on the Rotor-Gene Q 5plex instruments from QIAGEN (5plex and 5plex HRM, cat. no. 9002033 or 9002032) or the Mic qPCR cycler (Mic 4-Channel and Mic and IVD, cat. no. MIC-4 or MIC-IVD) from Bio Molecular Systems. Other real time PCR machines can be used only after validation of the optimal PCR conditions and settings.
- MicPCR Software (Bio Molecular Systems)
- PreCursor-M AnOGYN MIC v1.0.micemplate

Warnings and Precautions

For Research Use Only.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs).



PreCursor-M AnoGYN master mix: Danger! Suspected of damaging fertility or the unborn child. Wear protective gloves, protective clothing, eye protection and face protection. If exposed or concerned: get medical advice. Store locked up. Dispose of contents and container to an approved disposal plant.

General precautions

Use of PCR tests requires good laboratory practices that are dedicated to molecular biology, including maintenance of equipment, that are dedicated to molecular biology and is compliant with applicable regulations and relevant standards. Always pay attention to the following:

- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Prevent microbial and nuclease (DNase) contamination of the specimen and the kit. DNase may cause degradation of the DNA template.
- Sample preparation and PCR set up should be performed in a dedicated area separated from the area in which PCR amplification will be performed. To minimize the risk for PCR reaction contamination the use of a PCR-cabinet with UV-radiation is strongly recommended.
- The working area needs to be kept clean especially from PCR contaminants. Cleaning can be performed with 0.1 N HCl and/or UV-radiation, or by a user preferred and proven method.
- Include a no template control in your PCR experiments to confirm that reagents are free of contamination.
- Avoid DNA or PCR product carryover contamination, which could result in a false-positive signal.
- Use extreme caution to prevent contamination of the mixes with the synthetic materials that are contained in the PCR reagents.
- Always use DNase-free disposable pipet tips with aerosol barriers.
- Reagents of the PreCursor-M AnoGYN are optimally diluted. Do not dilute reagents further as this may result in a loss of performance.
- All reagents supplied in the PreCursor-M AnoGYN kit are intended to be used solely with the other reagents supplied in the same kit. Do not substitute any reagent from one kit with the same reagent from another PreCursor-M AnoGYN kit, even from the same batch, as this may affect performance.
- Refer to the Rotor-Gene Q or Mic qPCR user manual for additional warnings, precautions, and procedures.
- Alteration of incubation times and temperatures may result in erroneous or discordant data.
- Do not use components of the kit that have passed their expiration date, or that have been incorrectly stored.
- Minimize the exposure of components to light; reaction mixes may be altered due to exposure.
- Discard sample and assay waste according to your local safety procedures.

Reagent storage and handling

PreCursor-M AnoGYN is shipped on dry ice. Upon receipt store immediately at -30 to -15°C in a constant temperature freezer and protected from light. It is advised to store the kit in a laboratory that is free of PCR amplicons. When stored under the specified storage conditions, PreCursor-M AnoGYN is stable until the expiration date stated on the box label. Once opened, reagents can be stored in their original packaging at -30 to -15°C .

Repeated thawing and freezing should be avoided. Do not exceed a maximum of 3 freeze–thaw cycles. Before use, thaw the reagents by placing them at room temperature for 15 minutes. Mix by gently vortexing and centrifuge the tubes before opening.

Sample preparation and handling

The PreCursor-M AnoGYN PCR is performed on bisulfite converted DNA derived from human samples, for instance formalin-fixed paraffin-embedded anal biopsies or anal swab specimens. The protocol requires DNA Extraction, DNA concentration measurement, followed by aliquoting of optimal eluate volume before starting with the bisulfite-conversion protocol. The kit is compatible with the EZ DNA Methylation™ Kit and the EZ DNA Methylation Lightning™ from ZYMO Research (cat.no. D5001/D5002 or cat.no. D5030/D5031). We recommend the following workflow and methods:

- DNA extraction
Standard DNA extraction kits (e.g. column-based and magnetic bead-based kits) are compatible with the PreCursor-M AnoGYN (e.g. QIAamp DNA mini kit or QIAamp DNA FFPE Tissue kit for formalin-fixed paraffin-embedded specimens).
- DNA concentration measurement
Prior to bisulfite-conversion of DNA, measure DNA concentration. Suitable systems for measuring the DNA concentrations are Qubit® Fluorometer, NanoDrop 3300 Fluorospectrometer (both from Thermo Fisher Scientific) or equivalents.
- Aliquoting DNA eluate
Recommended DNA input for bisulfite-conversion is 200 ng. Elution after bisulfite-conversion is in 10 μL elution buffer. DNA concentration input for bisulfite-conversion can range from 100 ng to 2 μg for valid test results. If DNA concentration is too low for bisulfite-conversion, repeat the DNA extraction with a higher input volume of the clinical sample or elute DNA in a smaller elution volume. The optimal input in the PCR is 50 ng of converted DNA.
- Bisulfite-conversion
Bisulfite-conversion with EZ DNA Methylation Kit and EZ DNA Methylation Lightning Kit is performed according to the manufacturer's recommendations.
Note: maximum amount of sample DNA should not exceed 2 μg to obtain a sufficiently high conversion-efficiency (>98%).

General recommendations for bisulfite-conversion

The bisulfite-conversion reaction should be performed in a designated area separate from where the PreCursor-M AnoGYN Master Mix is stored and dispensed, to avoid contaminating the reagents.

The input in the PreCursor-M AnoGYN reaction is 5 μ L of bisulfite-converted DNA.

If the internal sample quality control is negative (i.e., ACTB C_T values are >30), the specimen bisulfite-converted DNA preparation resulted in material of insufficient quantity and/or quality and is considered invalid. To reach an ACTB C_T that is within the valid range repeat bisulfite-conversion reaction with a higher input of specimen DNA and/or repeat DNA isolation with a higher input of specimen.

Bisulfite-converted DNA can be stored up to 24 hours at 2–8°C and up to 3 months at –15°C to –70°C. Repeat freeze-thawing of the bisulfite-converted DNA should be avoided at all times. The number of freeze-thaw cycles should not exceed three, to maintain sufficient quality.

Procedure for the Rotor-Gene Q PCR machine

Important points before starting

- Take time to familiarize yourself with the Rotor-Gene Q 5plex instrument before starting the protocol. See the instrument user manual.
- Before the first run of the day, perform a warm-up run for Rotor-Gene Q 5plex instrument at 95°C for 10 minutes.

qPCR Reaction Setup

Up to 70 bisulfite-converted DNA samples can be tested within the same run besides a calibrator and no template control. In a designated DNA- and amplicon-free area:

1. Thaw the PreCursor-M AnoGYN master mix and calibrator completely.
2. Briefly vortex and spin down prior to pipetting.
3. Dispense 15 µL of ready-to-use PreCursor-M AnoGYN master mix into the appropriate tubes of the tube-strips (at maximum 72 tubes per Rotor-Gene-Q run). Reaction setup can be done at room temperature.
4. Return the vial with remaining PreCursor-M AnoGYN master mix to the freezer to avoid any material degradation.
5. Transfer tube strips to designated amplicon-free area.

In a designated amplicon-free area:

6. Add 5 µL of bisulfite-converted DNA to the appropriate tubes containing PreCursor-M AnoGYN master mix.
7. Add 5 µL of water for the No Template Control (NTC) reaction to tube position 2.
8. Add 5 µL of PreCursor-M AnoGYN Calibrator to tube position 1.
9. Close the tubes by pressing the caps on the tubes.
Note: The PCR tubes can be stored up to 30 minutes between pipetting samples into the PCR tubes and start of the experiment in the machine at 2-8°C in the dark.
10. Return the PreCursor-M AnoGYN Calibrator to the freezer to avoid any material degradation.
11. Transfer tube strips to designated PCR amplification area.

In a designated PCR amplification area:

12. Place the tube strips into 72-well Rotor, empty spaces should be filled with empty or dummy tube strips with caps as counterweight.
13. Lock the tubes by attaching the locking ring and place the Rotor in the Rotor-Gene-Q machine.
14. Close the lid of the Rotor-Gene-Q machine.

Rotor-Gene Q PCR machine set up

15. Start the Rotor-Gene-Q Series Software.
16. Select Rotor type: '72-well rotor' and 'Locking ring attached' and click next-button.
17. At operator, enter initials, click next button.
18. At volume enter 20 µL and click next-button.

19. Click 'Edit profile'-button and define the real-time PCR cycling conditions as follows:

Temperature	Time	Cycles	Acquiring Fluorescence
95 °C	5 minutes	1	No
95 °C	15 seconds	40	No
60 °C	60 seconds		Yes*

*Acquiring to cycling A on Green, Orange, and Yellow
Green= ZNF582; Orange= β -actin; Yellow=ASCL1

20. Click OK and then click 'Gain optimization'.
21. Click 'Optimise Acquiring'. Check tube position is set to position 1 for all dyes (e.g. Gain optimization must be performed on the Calibrator reaction tube).
22. Check the box for 'Perform optimisation before 1st acquisition'.
23. Click 'Close' followed by 'Next'.
24. Click 'Start run'
Optional: Click "Save Template" to add the run template to the list.
25. Click 'Edit samples' to enter sample names etc. (this can also be done after the run is completed).

Procedure for the Mic qPCR cycler

Important points before starting

- Take time to familiarize yourself with Mic qPCR instrument before starting the protocol. See the instrument user manual.

qPCR Reaction Setup

In a designated DNA and amplicon free area:

1. Thaw the PreCursor-M AnoGYN master mix and calibrator completely and protect the PreCursor-M AnoGYN master mix from light whenever possible.
2. Briefly vortex and spin down prior to pipetting.
3. Dispense 15 μ L of ready-to-use Precursor-M AnoGYN master mix into the appropriate tubes of the tube strips (at maximum 48 tubes per run). Reaction setup can be done at room temperature.

Note: Each tube is preloaded with silicone oil to prevent evaporation and condensation. Make sure you pipet tip does not touch the silicone oil.

4. Return the vial with remaining PreCursor-M AnoGYN master mix to the freezer to avoid any material degradation.
5. Transfer tube strips to designated amplicon-free area.

In a designated amplicon free area:

6. Add 5 μ L of bisulfite-converted DNA to the appropriate tubes containing PreCursor-M AnoGYN master mix.
7. Add 5 μ L of water for the No Template Control (NTC) reaction to tube position 2.
8. Add 5 μ L of PreCursor-M AnoGYN Calibrator to tube position 1.
9. Each time when 4 tubes are filled, close the tubes by using the Mic capping tool.

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

10. Return the PreCursor-M AnoGYN Calibrator to the freezer to avoid any material degradation.
11. Transfer tube strips to designated PCR amplification area.

In a designated PCR amplification area

12. Place tube strips into 48-well rotor according to the assigned positions, unused positions should be filled with dummy tube strips holding 20 μ L of water with caps as counterweight.
13. Attach locking ring.

Mic qPCR machine set up

14. Start the micPCR Software.
15. Click New, Run from Template
16. Select template: Precursor-M AnoGYN v1.0 and click Ok.

17. Select Samples and enter the sample identification number(s) into this list. The Calibrator is always on position 1 and the NTC on position 2. The list represents the expected layout of the rotor. Sample identification number(s) can also be added after the run is completed.
18. Click on the Mic instrument icon (right top).
19. Click Start Run.
20. Enter the run (experiment) name and click Save.
21. Click Start. The Precursor-M AnoGYN run should start.

Interpretation of results

Acquiring C_T values

For the Rotor-Gene Q PCR machine

1. After the run is completed go to 'Analysis tool window', select 'Cycling A. Green', and click 'OK', then select 'Cycling A. Orange', and click 'OK', and finally select 'Cycling A. Yellow', and click 'OK'
2. Enter the analysis settings for the three dyes as follows:

Color	Target	Threshold	Slope correction	Take off adjustment
Orange	ACTB	0,04	Yes	9 to 40
Green	ZNF582	0,03	Yes	9 to 40
Yellow	ASCL1	0,04	Yes	9 to 40

Note: these analysis settings can be saved for each detection channel in an analysis template for next runs.

3. Select the wells to be used for analysis.
4. Check the fluorescence data for each reaction for each dye for typical amplification curves (logarithmic increase of fluorescence). If the baseline does not provide satisfactory results, the sample can be repeated and interpret the results accordingly.
5. Save the run as an 'Excel analysis sheet' (located under 'File' in toolbar) to export the results as an Excel CSV file.

For the Mic qPCR machine

1. After the run is successfully completed click OK.
2. Click on save.
3. If the sample ID's haven't been added yet, fill in the sample identification number(s).
4. Go in the left pane to Analysis and click on 'Cycling +'. Select target ZNF582.
5. Go in the left pane to Analysis and click on 'Cycling +'. Select target ASCL1.
6. Go in the left pane to Analysis and click on 'Cycling +'. Select target β -Actin.
7. The fluorescent signals in the 3 detection channels (Green, Yellow and Orange) are automatically analysed according to their channel specific settings.
8. Check the fluorescent data in each detection channel for each reaction for typical logarithmic amplification curves (i.e., logarithmic increase of fluorescence). If the amplification curve(s) is/are not satisfactory, repeat the sample with the PreCursor-M AnOgYN or contact support.
9. To export go to the left pane and click on 'Reports +'. A preview of the report will be displayed.
10. Click on the icon 'Export' (top of right pane) and select export format 'XLSX' and click OK.

Validation criteria for successful analysis

1. The PCR run is valid when the controls comply with below criteria for all targets.

	ACTB C _T	ZNF582 C _T	ASCL1 C _T
NTC	>40 (no Ct value)	>40 (no Ct value)	>40 (no Ct value)
Calibrator	<30	<30	<30

2. The C_T value of *ACTB* of a sample should be ≤ 30 for a valid result.

Calculating ΔΔCt values

3. Use the below formula to calculate ΔΔCt values of the target genes *ZNF582* and *ASCL1*. Lower ΔΔCt values correspond with higher level of methylation of the target gene.

$$\Delta\Delta C_{t_{\text{target gene}}} = (C_{t_{\text{target gene sample}}} - C_{t_{ACTB \text{ sample}}}) - (C_{t_{\text{target gene calibrator}}} - C_{t_{ACTB \text{ calibrator}}})$$

4. *Optional: the methylation level of a target can also be expressed as 'ΔΔCt ratio' (i.e. 2^{-ΔΔCt}) for interpretation using an incremental increasing scale starting at 0, in which 0 indicates no methylation and increasing numbers indicate higher methylation levels⁹. See the formula below:*

$$2^{-\Delta\Delta C_{t_{\text{target gene}}}} \times 100$$

Troubleshooting

This troubleshooting guide may be helpful in solving any problems that may arise. If the problem persists or in case of questions or other information contact your local distributor or Self-screen at info@self-screen.nl.

Comments and suggestions	
Sample DNA concentration too low for bisulfite conversion	
Check DNA extract	Repeat DNA extraction with more concentrated clinical sample
Sample is scored invalid: the amplification of β-actin is too low or absent	
a. Pipetting error or omitted reagents	Check pipetting scheme and the reaction setup. Repeat the PCR run.
b. Check the DNA concentrate	Increase DNA input for bisulfite-conversion to a maximum. Bisulfite-conversion reaction has optimal performance for DNA input ranging from 100 ng–2 μ g
c. Check the bisulfite-converted eluate	Repeat bisulfite conversion, when necessary a higher DNA input can be used.
Positive control is scored invalid: the amplification is too low or absent for one or more of the targets	
b. Pipetting error or omitted reagents	Check pipetting scheme and the reaction setup. Repeat the PCR run.
c. Partial degradation	Store kit contents at –30 to –15°C. Avoid repeated freezing and thawing to a maximum of three cycles.
d. PCR reagents partially degraded	Store kit contents at –30 to –15°C and keep the reaction mixes protected from light. Avoid repeated freezing and thawing.
e. Strip tube inversion	Check the pipetting scheme and the reaction setup.
f.	
g. Expiry date	Check the expiry date of the used kit.
h. Time-delay between pipetting samples and start of the run	PCR reactions mixes can be stored at 2–8°C for 30 minutes in the dark between dispensing samples into the PCR reactions and starting the run in the machine.
No template control (NTC) is invalid	
i. Pipetting error	Check pipetting scheme and the reaction setup. Repeat the PCR run.
j. Cross-contamination	Replace all critical reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carryover contamination.
k. Reagent contamination	Replace all critical reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carryover contamination.
l. Strip tube inversion	Check the pipetting scheme and the reaction setup.
m. Probe degradation	Keep reaction mixes protected from light. Check for false positive on the fluorescence curve.
Absent or low signals in sample, but the control run ok	
n. Inhibitory effects	Always check there are no remains of buffer on the filter after centrifugation during bisulfite-conversion. Repeat bisulfite-conversion.
o. Pipetting error	Check pipetting scheme and the reaction setup. Repeat the PCR run.

Limitations















- PreCursor-M AnoGYN reagents are for Research Use Only.
- Use of PCR tests requires good laboratory practices, including maintenance of equipment, that are dedicated to molecular biology and is compliant with applicable regulations and relevant standards.
- The PreCursor-M AnoGYN is to be used by laboratory professionals trained in the use of the Rotor-Gene Q cyclers or Mic qPCR cyclers.
- Strict compliance with the user manual (handbook) is required for optimal PCR results.
- Do not use components beyond their expiration date.
- All reagents supplied in the PreCursor-M AnoGYN are intended to be used solely with the other reagents supplied in the same kit. This may otherwise affect performance.
- Any off-label use of this product and/or modification of the components will void Self-screen B.V.'s liability.
- It is the user's responsibility to validate system performance for any procedures used in their laboratory that are not covered by the Self-screen performance studies.

References

1. Costello, J.F., and Plass, C. (2001) Methylation matters. *J. Med. Genet.* 38, 285–303.
2. Steenberg, R.D.M. et al. (2014) Clinical implications of (epi)genetic changes in HPV-induced precancerous lesions. *Nat. Rev. Cancer* 14, 395–405.
3. van der Zee, R.P. et al. (2019) Host Cell Deoxyribonucleic Acid Methylation Markers for the Detection of High-grade Anal Intraepithelial Neoplasia and Anal Cancer. *Clin Infect Dis.* 68(7):1110-1117.
4. van der Zee, R.P. (2021) Cancer Risk Stratification of Anal Intraepithelial Neoplasia in Human Immunodeficiency Virus-Positive Men by Validated Methylation Markers Associated With Progression to Cancer. *Clin Infect Dis.* 72(12):2154-2163.
5. van der Zee, R.P., (2021). Characterisation of anal intraepithelial neoplasia and anal cancer in HIV-positive men by immunohistochemical markers p16, Ki-67, HPV-E4 and DNA methylation markers. *Int J Cancer.* 149(10):1833-1844
6. van der Zee, R.P. (2021) DNA methylation markers have universal prognostic value for anal cancer risk in HIV-negative and HIV-positive individuals. *Mol Oncol.* 15(11):3024-3036.
7. Voss, F.O. (2023) Clinical validation of methylation biomarkers for optimal detection of high-grade vulvar intraepithelial neoplasia. *Int J Cancer.* 153(4):783-791.
8. Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25, 402–8.
9. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 2008; 3:1101–8.

Symbols

The following symbols may appear on the packaging and labelling:

Symbol	Symbol definition
	Use by
	Contains reagents sufficient for <N> reactions
	Catalogue number
	Lot number
	Material number
	Components
	Contains
	Number
Rn	R is for revision of the Instructions for Use (Handbook) and n is the revision number
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Protect from light
	Consult instructions for use
	Caution

Contact information

For technical assistance and more information, please contact Self-screen or our local distributors.

Manufacturer: Self-screen B.V., Plesmanlaan 125, 1066CX, Amsterdam, The Netherlands.

info@self-screen.nl

Revision History

Revision	Changes
1	First version