



**Mitomic<sup>®</sup>**

# Endometriosis Test Real-Time PCR (MET<sup>™</sup>) Kit

REF 200-01

Instructions for use

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For use with QuantStudio<sup>™</sup> 5 Real-Time  
PCR System or Bio-Rad CFX96 Touch<sup>™</sup>  
Real-Time PCR detection System



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## Intended Use

The Mitomic® Endometriosis Test Real-Time PCR (MET™) kit – RUO is a qualitative real-time polymerase chain reaction (PCR) assay for the detection of an 8.7 kb mitochondrial DNA deletion and a 18S rRNA nuclear DNA sequence, in human plasma samples. The MET™ assay must be used in combination with the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (96 wells, 0.2 mL) or the Bio-Rad CFX96 Touch™ Real-Time PCR Detection System (96 well, 0.2ml).

The Mitomic® Endometriosis Test is intended for Research Use Only. Not for use in diagnostic procedures.

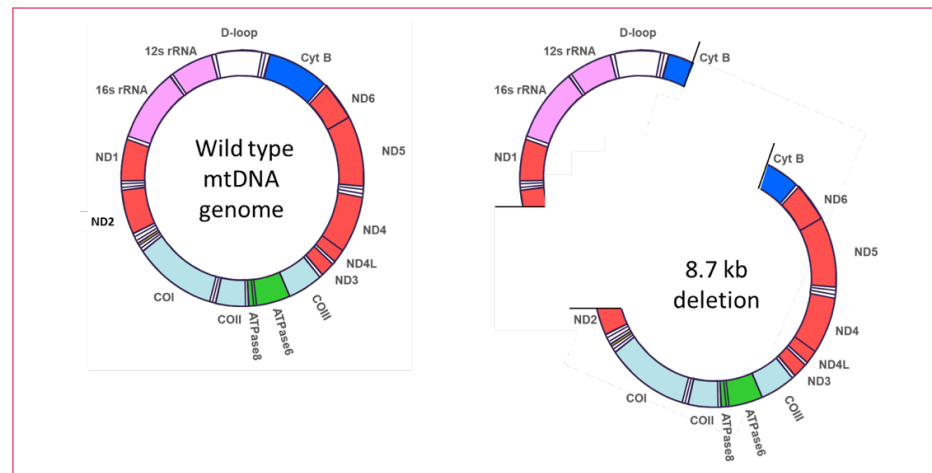
## Summary and Explanation

Endometriosis is characterized by the abnormal deposition of endometrial tissue outside the uterus in 5% to 10% of females of childbearing age<sup>1,2</sup>. Several symptoms can be associated to endometriosis, such as pelvic pain, dysmenorrhea or subfertility, but are often insufficient to diagnose endometriosis: the diagnostic gold standard being laparoscopic surgery. Misinterpretation of symptoms as well as postponement of surgery are delaying the diagnosis and treatment for endometriosis, negatively impacting quality of life.

MDNA Life Sciences identified a novel 8.7 kb mitochondrial DNA deletion (Figure 1), associated with endometriosis<sup>3</sup>. The mitochondrial DNA deletion encompasses all or part of the genes NADH dehydrogenase subunits 2 (ND2) to the cytochrome b generating two sublimons, that re-circularize to form new detectable sequences distinct from wild-type mitochondrial sequence.

The 8.7 kb mitochondrial deletion is detectable in endometriosis lesions and circulating blood and its abundance correlates with the presence of endometriosis<sup>3</sup>. Indeed, similarly to previously reported mitochondrial deletions<sup>4</sup>, females with high level of the 8.7 kb deletion have a higher risk of a subsequent surgical diagnosis of endometriosis than do those with lower levels of the deletion.

FIGURE 1: SCHEMATIC REPRESENTATION OF THE HUMAN MITOCHONDRIAL GENOME, WITH THE 8.7 KB DELETION BREAKPOINTS



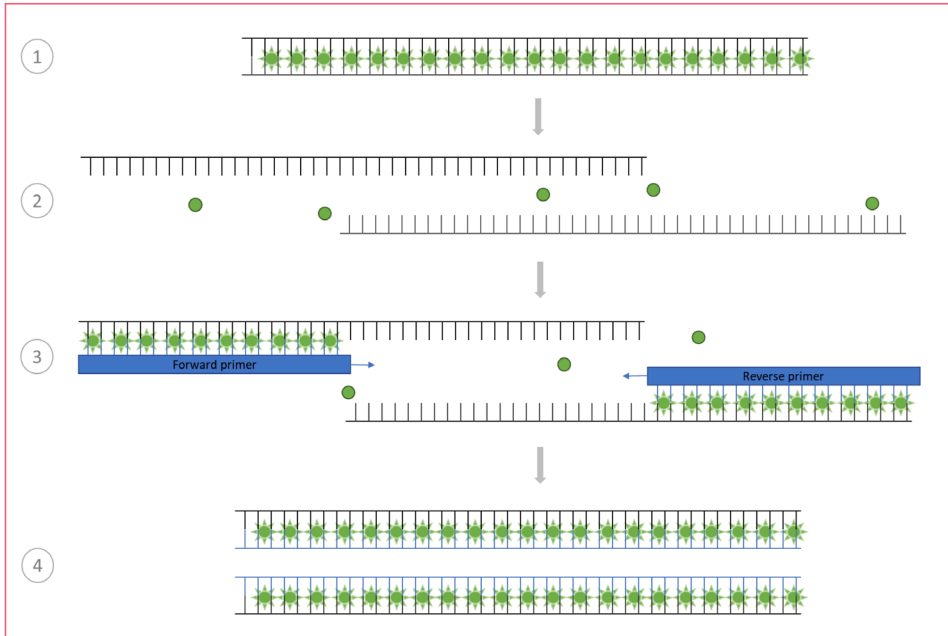
## Principle of the Mitomic® Endometriosis Test

The Mitomic® Endometriosis test is a real time polymerase chain reaction (qPCR) assay, designed to detect an 8.7 kb mitochondrial DNA deletion associated with endometriosis, in DNA extracted from plasma samples. The abundance of the deletion is then normalized using the level of the 18S rRNA genomic control. The ratio of these targets defines a MET™ score that is then adjusted with a calibrator.

The Mitomic® Endometriosis Test exploits the principle of the SYBR® Green I Dye: an intercalating dye that fluoresces when it binds to double-stranded DNA. As described in Figure 2, when SYBR® Green I Dye is added to a sample, it immediately binds to the double-stranded DNA present in the sample (1) and fluoresces. During the qPCR, DNA templates are denatured, SYBR® Green I Dye released from DNA templates, and its fluorescence reduced (2). During the qPCR extension step, forward and reverse primers hybridize to specific sequences and the SYBR® Green I Dye binds to the double-stranded DNA that is being amplified (3). As the qPCR progresses, more double-stranded DNA amplicons are produced and bound to the SYBR® Green I Dye, leading to a fluorescence intensity that is proportional to the amount of qPCR products (4).

The use of real-time PCR (qPCR) allows results to be analyzed without post-PCR processing.

FIGURE 2 SYBR® GREEN REACTION PRINCIPLE



Legend: Fluorescing SYBR Green molecules are represented by green stars; Non-fluorescing SYBR green molecules, by green circles; Primers, by blue boxes; DNA strand (plus), by black comb; DNA strand (minus), by gray comb.

## MET™ assay workflow

No dedicated training to the MET™ workflow is required. For technical support, contact the technical support: [techsupport@mdnalifesciences.com](mailto:techsupport@mdnalifesciences.com)

The MET™ assay can be divided into three major steps (Figure 3):

1. DNA extraction from human plasma samples
2. Target detection by qPCR
3. MET™ results / Data interpretation

FIGURE 3 MET™ ASSAY GLOBAL WORKFLOW.



\*DNA is extracted either manually or automatically.

## DNA extraction from plasma samples

DNA extractions are performed with either the QIAamp® DNA Mini Kit (QIAGEN® Cat #51304 or #51306) or the QIAcube® 96 DNA QIAcube HT (QIAGEN® Cat. #51331 (Figure 3)). **Important note: The MET™ assay is not compatible with the QIAamp DNA Blood Mini Kit (QIAGEN® Cat #51104 or #51106).** To monitor sample contamination, it is required to perform a No Extraction Control (NEC: use elution buffer as input instead of plasma) in parallel to the extraction of the plasma samples.

### Target detection by qPCR

After DNA extraction, qPCR plates are prepared with the MET™ kit reagents, controls (Positive control/Calibrator, No Template Control (NTC) and No Extraction Control (NEC)) and the extracted DNA. PC/Calibrator and NTC are controls required to validate the qPCR runs. NEC monitors sample contamination during DNA extraction and should be tested for every new round of DNA extraction. The 18S rRNA target is assessed in triplicate, while the 8.7 kb deletion target is measured in triplicate. qPCR runs are performed either on the QuantStudio™ 5 Real-Time PCR System (96 wells, 0.2 mL), or on the CFX96 Touch™ Real-Time PCR Detection System (see Figure 3).

### MET™ results / Data interpretation

A valid MET™ assay results in a MET™ score. MET™ scores are defined by the ratio between the targeted mitochondrial deletion and a control region, the 18S rRNA genomic region. For this purpose, the quantitation cycle (Cq) of the mitochondrial deletion, performed in triplicate, is normalized with the 18S rRNA signal. Once normalized, this MET™ score is further calibrated with a score, assigned specifically to the PC/calibrator lot (see section “Data interpretation” in “Quality checks and data interpretation” for more information).

## Material and equipments

### Provided material

The contents of the Mitomic® Endometriosis Test Real-Time PCR Kit are sufficient for 21 samples (96 reactions) in one run or 7 samples (40 reactions) in three runs.

### Kit Contents

Mitomic® Endometriosis Test Real-Time PCR Kit		
Catalog number		200-01
Number of reactions		96
Number of samples		21
Color	Identity	Volume
Green	MET™ PCR Master Mix	1 x 1380 µl
Blue	MET™ 8.7 kb DEL primers	1 x 100 µl
Red	MET™ 18S rRNA primers	1 x 40 µl
Yellow	MET™ PC/Calibrator	1 x 70 µl
Black	MET™ NTC	1 x 70 µl
Clear	MET™ PCR Water	1 x 550 µl

### MET™ PCR Master Mix

The MET™ PCR Master Mix component contains all reagents needed for the amplification of both targets, except primers and template, i.e. italicize *Taq*, reaction buffer, MgCl<sub>2</sub>, dNTPs, and the SYBR® Green I Dye.

### MET™ 8.7 kb DEL primers

MET™ 8.7 kb DEL primers contains forward and reverse primers for the amplification of the 8.7 kb deletion sequence. It is ready-to-use, no dilution required.

### MET™ 18S rRNA primers

MET™ 18S rRNA primers contains forward and reverse primers for the amplification of the 18S rRNA sequence. It is ready-to-use, no dilution required.

### MET™ PC/Calibrator

The MET™ PC/Calibrator is a mix of plasmids containing the 8.7 kb DEL and 18S rRNA sequences. It is first employed as a Positive Control to validate each qPCR run, i.e. its amplification ensures that all reagents of the qPCR mix are working properly, that the thermocycler is set up correctly, and the reagents were stored in adequate conditions. Secondly, it is used as a Calibrator. Each Calibrator has an assigned value that is used to determine the Calibration Factor for each qPCR run. The Calibration Factor is added to the MET™ score of each sample to obtain a final MET™ score (see section Quality Checks & Interpretation of Data Generated for more details).

## MET™ NTC

The MET™ No Template Control (NTC) does not contain target DNA and allows the detection of contamination of the PCR reagents. In the qPCR plate, the NTC reaction contains all real-time PCR components except the DNA template. Detection of a positive signal in an NTC reaction indicates the presence of contaminating nucleic acids.

## MET™ PCR water

The MET™ PCR Water is needed to adjust the pre-mix volumes to 15 µl for each target.

## Material required, but not provided\*

### Equipment, consumables and reagents for automated DNA extraction

- QIAcube® HT System (Qiagen, cat. no. 9001793) †
- QIAamp® 96 DNA QIAcube® HT Kit (Qiagen, cat. no.51331)
- QIAcube® HT Plasticware (Qiagen, cat. no.950067)
- Elution Microtubes RS (Qiagen, cat. no.120008)
- Reagent Trough with Lid 70 ml / 170 ml (Qiagen, cat. no. 990554 / 990556)

### Reagents for manual DNA extraction

- QIAamp® DNA Mini Kit (cat. no. 51304 or 51306)

### Equipment and consumables for real-time PCR

- QuantStudio™ 5 Real-Time PCR System (Applied Biosystem™, cat no. A28139) †
  - 96 well plate for QuantStudio™ 5 Real-Time PCR System (Applied Biosystems™ MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode, cat no. 4483354)
  - Sealing Foil (Applied Biosystems™ MicroAmp™ Optical Adhesive Film, cat no. 4360954)
- CFX96 Touch™ Real Time PCR Detection System (Bio-Rad, cat no. 1855195) †
  - 96 well plate for CFX96 Touch™ Real Time PCR Detection System (Bio-Rad Hard-Shell® 96W Low Skirted White shell/Clear wells, cat no. HSP9601)
  - Sealing Foil (Bio-Rad Microseal 'B' adhesive seal, cat no. MSB1001)

## General laboratory reagents and equipment

- Non-denaturing Ethanol, molecular biology grade, 96%-100%
- Isopropanol (for buffer ACB preparation in QIAcube® HT kit)
- Pipets 5-1000 µL (adjustable) and sterile, DNase/RNase-free pipet tips with filters †
- Vortex mixer
- Benchtop centrifuge with rotor for 2 ml reaction tubes
- Centrifuge with rotor for qPCR plates
- Centrifuge with swing-out bucket rotor for blood collection tubes
- Fridge (+2°C - +8°C) †
- Freezer (-30°C - -15°C) †
- Surface decontaminant DNA™ AWAY (Fisher Scientific, cat no. 7010) 990556

\*Recommended chemicals and/or equipment are potentially hazardous. Ensure that appropriate personal protective equipment is worn and that suitable protective measures are taken before use.

†Ensure that equipment is clean, properly maintained and calibrated according to the manufacturer's recommendations.



## Warnings and Precautions

### Safety information

Universal safety precautions must always be followed. Patient samples should be treated as potentially hazardous and handled accordingly. Samples and assay waste must be discarded according to your local safety regulations.

When working with chemicals, suitable lab coat, protective disposable powder-free gloves and goggles should always be worn. For more information, consult the MET™ Product Safety Information Sheet (PSIS). Product Safety Information Sheets are available online ([www.mdnlifesciences.com/mitomic-tests](http://www.mdnlifesciences.com/mitomic-tests)).

## General precautions

### Attention must be paid to the following:

- Incorrectly stored or transported reagents and MET™ kits should not be used. Recommended storage and shipping conditions are written on product labels.
- Expiration date, printed on boxes and labels, of every reagent or kit component must be noted. Expired reagents and MET™ kits should not be used.
- Assign dedicated working areas for sample preparation, reaction setup and amplification/detection activities: the workflow of the laboratory evolving unidirectionally. Disposable gloves must be worn in each working area and changed before entering another one.
- Equipment must be dedicated to specific working areas and not moved from one area to another.
- Equipment must be clean and its maintenance up to date.
- Blood sample must be collected in 10ml K2-EDTA Vacutainer® tubes (BD Life Sciences Cat no 367839) and filled according to the manufacturer recommendations. Strict adherence to the plasma preparation protocol must be followed.
- Patient samples must be stored separately from kit components.
- Samples must be handled methodically to ensure correct identification and traceability.
- Caution must be observed to ensure correct sample testing: sample recording, sample tracking and pipetted volumes and reagents.
- It is recommended to proceed continuously with the MET™ workflow, without stopping in between steps.
- Aliquoting the QIAamp® DNA Mini Kit components and using new aliquots per DNA extraction round is recommended before the first use.
- Repeated freeze/thaw cycles of the DNA eluate should be avoided, as this may reduce the assay performance.
- Use of qPCR tests require good laboratory practices, dedicated to molecular biology and compliant with applicable regulations, samples traceability and relevant standards.
- The MET™ Master Mix should be protected from light.
- MET™ components should be homogenized after thawing (pipetting up and down or pulse-vortexing), followed by a brief centrifugation. No foam or bubbles should be present in the reagent tubes.
- Components from kits having different lot numbers must not be mixed.
- Tubes must be kept closed whenever possible during hands-on steps to limit contamination.
- Microbial and nuclease (DNase/RNase) contamination of samples and kit components must be avoided.
- DNase/RNase-free disposable pipet tips with aerosol barriers must be used.
- Post amplification PCR plates should not be opened, to avoid carry-over contamination.
- Although not mandatory, the use of protective sleeves may be useful to reduce contamination during the qPCR plate preparation.
- Additional controls may be tested, according to guidelines, requirements of local, state and/or federal regulations or accrediting organizations.

## Shipping and storage

The Mitomic® Endometriosis Test Real-Time PCR kit is shipped on dry ice. Contact the local distributor if the outer packaging has been damaged during transit or reagents are thawed.

The Mitomic® Endometriosis Test Real-Time PCR kit should be stored immediately at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  upon receipt, away from light. MET™ components are stable until the expiration date stated on the kit label. The in-use stability is 9 months after opening.

Repeated freeze/thaw cycles (>6 x) may reduce the assay sensitivity.

## MET™ Procedures

### Sample Handling, Storage and Preparation

The Mitomic® Endometriosis Test assesses only DNA samples extracted from plasma samples. Plasma must be obtained from blood samples collected in **10ml K2-EDTA tubes**.

#### Blood collection

Whole blood is collected into commercially available **10 ml K2-EDTA Vacutainer®** tubes (BD Life sciences Cat no 367839), by filling the container to the target volume. **An incomplete filling increases the K2-EDTA concentration in the blood sample and interferes with MET™ assay results.** Following collection, the specimens must be maintained chilled (between  $2-8^{\circ}\text{C}$ ), and plasma preparation should be performed within 4 hours. This assay is compatible with fresh blood samples only, blood samples should never be frozen.

#### Plasma samples - plasma preparation from fresh blood sample

The plasma must be obtained from fresh whole blood (frozen blood is not accepted). The separation must be performed as follows:

- Centrifuge the 10ml Vacutainer without opening the tube for 10 minutes at  $2500 \times g$ , using preferably a refrigerated centrifuge set to  $4^{\circ}\text{C}$ , with a swing-out bucket rotor. The brake should be on.

- Immediately transfer the plasma (supernatant) into DNase/RNase-free polypropylene tubes. **4 ml of plasma should be transferred carefully to another tube by taking only the clear fluid (upper phase) and avoiding disturbing the buffy coat layer. Buffy coat contamination will interfere with the MET™ assay results.**
- Plasma samples can be stored at  $-20^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$ , if not used right away.

#### Fresh isolated plasma

- Transported at  $2^{\circ}\text{C}-8^{\circ}\text{C}$ , for less than 24 hours before DNA extraction.
- Stored at  $2^{\circ}\text{C}-8^{\circ}\text{C}$ , for less than 24 hours before DNA extraction.
- May be stored at  $-90^{\circ}\text{C}$  to  $-65^{\circ}\text{C}$  for long-term storage, or  $-15^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  for shorter term storage (ie. up to 4 weeks prior to DNA extraction).
- Labeled, handled and stored, according to local procedures.

#### Frozen isolated plasma

- Transported on dry ice.
- May be stored at  $-90^{\circ}\text{C}$  to  $-65^{\circ}\text{C}$  for long-term storage, or  $-15^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  for shorter term storage (ie. up to 4 weeks prior to DNA extraction).
- Labeled, handled and stored, according to local procedures.

#### Frozen plasma samples are thawed and prepared according to the following instructions:

- Place frozen plasma aliquots in a cold block and let them thaw at  $4^{\circ}\text{C}$ .
- Transfer plasma samples in the cold block, in a Class II biosafety cabinet.
- Mix thawed plasma samples by inverting the tubes 5 times.
- Frozen plasma must not be thawed more than once. Multiple freeze/thaw cycles lead to protein denaturation and precipitation, reducing potentially the recovery yields of circulating cell-free DNA.

#### DNA samples

- Extracted DNA may be stored at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  or  $-90^{\circ}\text{C}$  to  $-65^{\circ}\text{C}$ , for long-term storage.
- Labeled, handled and stored, according to local procedures.

## Protocol 1A: DNA isolation using the QIAamp® DNA mini kit

The following protocol is excerpted from the QIAGEN® handbook “QIAamp® DNA Mini and Blood Mini Handbook, #1102728, May 2016. **Important note:** The MET™ assay is not compatible with the QIAamp DNA Blood Mini Kit (QIAGEN® Cat #51104 or #51106). DNA extractions must follow the manufacturer recommendations written in the QIAamp® DNA Mini Kit Handbook, with an input of 200 µl and an elution with 200 µl of AE buffer. At the elution step, incubate the columns at room temperature (15 °C – 25 °C) for 5 min with the Buffer AE, prior to centrifugation.

### Buffer preparation

- **Buffer AL:** (Room temperature, 15 °C – 25 °C)
  - Mix Buffer AL thoroughly by shaking before use.
  - Buffer AL is stable for 1 year, when stored at room temperature (RT).
  - Note: Do not add Protease directly to Buffer AL.
- **Buffer AW1:** (Room temperature, 15 °C – 25 °C)
  - Buffer AW1 is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) to AW1 concentrate, as indicated on the bottle.
  - Buffer AW1 is stable for 1 year, when stored closed at RT.
- **Buffer AW2:** (Room temperature, 15 °C – 25 °C)
  - Buffer AW2 is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) to AW2 concentrate, as indicated on the bottle.
  - Buffer AW2 is stable for 1 year, when stored closed at RT.

### Things to do before starting

- Equilibrate samples to room temperature (15 °C – 25 °C).
- Prepare a water bath or a heating block at 56 °C.
- Equilibrate Buffer AE to room temperature.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the manufacturer instructions.
- Check for precipitates in Buffer AL. If a precipitate has formed, dissolve it by incubating the buffer at 56 °C.
- Prepare and label every tube.
- Aliquot ethanol.

### Protocol

1. For each plasma sample and NEC, pipet 20 µl protease K solution in a 1.5 ml microcentrifuge tube.
2. Add 200 µl plasma to the microcentrifuge tube.

Note: A Negative Extraction Control (NEC) is required for each extraction run. For this purpose, add 200 µl Buffer AE instead of plasma to a microcentrifuge tube.

It is possible to add protease to samples that have been dispensed in microcentrifuge tubes. In this case, ensure proper mixing after adding the enzyme.

3. Add 200 µl Buffer AL to sample. Mix by pulse-vortexing for 15 s to get a homogeneous solution.
4. Spin the 1.5 ml microcentrifuge tubes down to remove drops from the lid.
5. Incubate at 56 °C for 10 min.

Note: DNA yield reaches a maximum with a lysis of 10 min at 56 °C. Longer incubation have no effect on yield or quality of the purified DNA.

6. Spin the 1.5 ml microcentrifuge tubes down to remove drops from the lid.
7. Add 200 µl ethanol (96%–100%) to the samples, mix again by pulse-vortexing for 15 s. After mixing, spin the 1.5 ml microcentrifuge tube down to remove drops from the lid.
8. Carefully apply the mixture from step 7, to the QIAamp® Mini spin columns (in a 2 ml collection tube), without wetting the rim. Close the cap, and centrifuge at 6000 x g for 1 min.

9. Place the QIAamp® Mini spin column in a clean 2 ml collection tube (provided with QIAGEN® kit) and discard the collection tube containing the filtrate.
10. Carefully open the QIAamp® Mini spin column and add 500 µl Buffer AW1, without wetting the rim. Close the cap and centrifuge at 6000 x g for 1 min.
11. Place the QIAamp® Mini spin column in a clean 2 ml collection tube (provided with QIAGEN® kit) and discard the collection tube containing the filtrate.
12. Carefully open the QIAamp® Mini spin column and add 500 µl Buffer AW2, without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g) for 3 min. Note the hinge position.
13. Recommended: Place the QIAamp® Mini spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed (20 000 x g) for 1 min, inverting the hinge position relative to step 11.

Note: This step helps to eliminate the risk of possible Buffer AW2 carryover.

14. Place the QIAamp® Mini spin column in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the filtrate. Carefully open the QIAamp® Mini spin column and add 200 µl of Buffer AE.
15. Incubate at room temperature (15 °C–25 °C) for 5 min, and then centrifuge at 6000 x g for 1 min.
16. Store DNA at -30 °C to -15 °C.

### Protocol 1B: DNA isolation using the QIAamp® 96 DNA QIAcube HT

The following protocol is excerpted from the QIAGEN® handbook “QIAamp® 96 DNA QIAcube® HT Handbook, #1080726, December 2013”. DNA extractions must follow the manufacturer recommendations written in the QIAamp® 96 DNA QIAcube® HT Handbook, with an input of 200 µl and an elution with 200 µl of elution buffer, provided in the kit.

Note: It is not recommended to perform the final step with the TopElute fluid.

Before proceeding with DNA samples, a visual inspection of the collected elution volumes and columns is recommended to search signs of clogging or other elution problems.

### Important points before starting

- Ensure that you are familiar with the operating instructions of the QIAcube® HT. Refer to the user manuals supplied with your instrument for a detailed protocol and possible error codes.
- Optional maintenance is not mandatory for instrument function but is highly recommended to reduce the risk of contamination or instrument malfunction.
- QIAcube® HT Prep Manager protocol files, that contain the information required to perform an extraction run on the QIAcube® HT instrument, are available at <https://www.qiagen.com/fr/shop/automated-solutions/sample-preparation/qiacube-ht/#orderinginformation>, under the Resources tab.



### Things to do before starting:

- Equilibrate reagents and samples to room temperature (15 °C – 25 °C).
- Check for precipitates in reagents. If a precipitate has formed, dissolve it by incubating the reagent at 37 °C with gentle shaking. Avoid vigorous shaking that may cause foaming.
- Check that Buffer ACB, Buffer AW1 and Buffer AW2 have been prepared according to the manufacturer instructions.
- Do a vacuum performance check to verify that all liquid has passed through the membrane.
- Ensure that the QIAcube® HT Prep Manager Software is installed and that the relevant version of the QIAamp® 96 DNA QIAcube® HT protocol is available on the computer.

### Protocol

1. Transfer 200 µl of plasma sample to the QIAcube® HT S-Block.

Note: A Negative Extraction Control (NEC) is required for each extraction run. For this purpose, transfer 200 µl of elution buffer instead of plasma to the QIAcube® HT S-Block.

2. Seal the S-block with a Tape Pad (supplied in the QIAamp® 96 extraction kit) and place it on ice while you set up the QIAcube® HT.
3. Turn on the QIAcube® HT.
4. Clean the worktable with a Surface Decontaminant (DNA™ AWAY) to eliminate DNA and DNase.

5. Turn the UV lamp on for 30 mins.
6. Ensure that there are sufficient numbers of tips for all steps, place boxes of QIAcube® HT filter tips in the indicated positions; make sure that the lids have been removed from the tip boxes and that the boxes are displayed as “available” on the software setup.
7. Load 2x 170 ml and 4x 70 ml reagent troughs in the appropriate positions, as shown on the software setup.
8. Open the setup wizard and select the appropriate number of columns, depending on the number of extractions to do.
9. Insert an extraction plate, after sealing any unused wells with the sealing film from the QIAcube HT plasticware kit.
10. Transfer the appropriate volumes of AW1, AW2, Ethanol, AE and ACB into the corresponding troughs, as indicated by the software.
11. Prepare the VXL + Proteinase k, as indicated in the table below, depending on the number of samples to extract and transfer into the appropriate trough. Make sure the solution is well homogenized.

Note: Be careful to prepare the mix for multiples of 8, as the instrument process a row of 8 troughs all at once. By example, if you perform 57 extractions, prepare the mix for 64 samples.

Sample	24	32	40	48	56	64	72	80	88	96
Buffer VXL (mL)	2.48	3.2	4	4.72	5.44	6.16	6.88	7.68	8.4	9.12
Proteinase K (µL)	620	800	1000	1180	1360	1540	1720	1920	2100	2280

12. Insert an elution plate into the QIAcube® HT and remove its lid.
13. Insert the S-block, take off the protective film, except for unused wells that need to be sealed.
14. Click start to begin the extraction.

Note: Before launching the extraction run, review the pre-report to ensure that the run settings correspond to those described in this protocol.

15. Follow the on-screen prompts.
16. At the end of the extraction run, place cap strips on used wells of the elution plate, then cover the plate with a lid and remove the elution plate from the QIAcube® HT.
17. At the workplace, remove the lid and the cap strips. Transfer the DNA sample into 1.5ml tubes.
18. Label your sample tube and store it at -20 °C.
19. Save the post-run report that is automatically generated by the software. The report includes a summary of the experiment (protocol used, volume input and output, plate layout, failure during the extraction...). Failures are listed and described in the “Exceptions” section of the report.
20. Wash the reagent troughs. Seal used S-Block and vacuum plate wells, those material can be used up to three times.
21. Clean the QIAcube® HT with a Surface Decontaminant (DNA™ AWAY).
22. Wash the removable tip chute and vacuum chamber.
23. Turn the UV lamp on for 30 mins.
24. Switch off the QIAcube® HT, following the on-screen cleaning instructions.
25. Perform maintenance as required by the manufacturer.

## Protocol 2: PCR assay set-up

The Mitomic® Endometriosis Test Real-Time PCR kit provides enough reagents for 21 samples, that could be assessed in up to 3 qPCR runs. The scheme in Figure 4 shows an example of a full plate experiment (21 samples) and in Figure 5, an example of a plate with 7 samples. Control and DEL targets are processed on the same qPCR plate. **An 18S rRNA target simplicate and an 8.7 kb deletion target triplicate are tested per sample.**

FIGURE 4 PLATE SET-UP FOR 21 SAMPLES

	1	2	3	4	5	6	7	8	9	10	11	12
A	01	09	17	01	01	01	09	09	09	17	17	17
B	02	10	18	02	02	02	10	10	10	18	18	18
C	03	11	19	03	03	03	11	11	11	19	19	19
D	04	12	20	04	04	04	12	12	12	20	20	20
E	05	13	21	05	05	05	13	13	13	21	21	21
F	06	14	NEC*	06	06	06	14	14	14	NEC	NEC	NEC
G	07	15	NTC	07	07	07	15	15	15	NTC	NTC	NTC
H	08	16	PC	08	08	08	16	16	16	PC	PC	PC

CTRL target
DEL target

FIGURE 5 PLATE SET-UP FOR 7 SAMPLES

	1	2	3	4	5	6	7	8	9	10	11	12
A	01	NEC		01	01	01	NEC	NEC	NEC			
B	02	NTC		02	02	02	NTC	NTC	NTC			
C	03	PC		03	03	03	PC	PC	PC			
D	04			04	04	04						
E	05			05	05	05						
F	06			06	06	06						
G	07			07	07	07						
H												

CTRL target
DEL target

## Things to do before starting

- Clean the workplace to ensure no template or nuclease contamination.

## Protocol

1. Thaw reagents at 4 °C (or at room temperature. Homogenize them by repeated up and down pipetting or quick vortexing). Spin down and keep them at 4 °C until use.
2. Prepare the pre-mixes as described in Tables 1 and 2, according to the number of samples to analyze. The volume calculations consider a final reaction volume of 20 µl and extra volumes to compensate pipetting error.
3. Dispense 15 µl of the 8.7 kb DEL and the 18S rRNA pre-mixes into the 96-well plate, according to the plate layout shown in Figure 4 or Figure 5.
4. Add 5 µl of DNA samples or controls (PC/Calibrator, NEC or NTC), according to the plate layout shown in Figure 4 or Figure 5, to reach a reaction a of 20 µl.

Note: Do not use smaller reaction volumes (reaction mix and sample) than 20 µl.

TABLE 1 PREPARATION OF THE 18S rRNA PRE-MIX

Component	1 reaction (µl)	Pre-mix 18S rRNA (µl) 7 samples + 3 Controls (10 + 1* rxn)	Pre-mix 18rRNA (µl) 21 samples + 3 Controls (24 + 3* rxn)
MET™ PCR Master Mix	10	110	270
MET™ 18S rRNA primers	1	11	27
MET™ PCR Water	4	44	108
Sample, PC/Calibrator	5	5 in each dedicated well	5 in each dedicated well
Total Volume	20	20 in each dedicated well	20 in each dedicated well

\*Extra volume of about 10%

TABLE 2 PREPARATION OF THE 8.7 KB DEL PRE-MIX

Component	1 reaction (µl)	Pre-mix 8.7 kb DEL (µl) 7 samples + 3 Controls (30 + 3* rxn)	Pre-mix 8.7 kb DEL (µl) 21 samples + 3 Controls (72 + 7* rxn)
MET™ PCR Master Mix	10	330	790
MET™ 8.7 kb DEL primers	1	33	79
MET™ PCR Water	4	132	316
Sample, PC/Calibrator	5	5 in each dedicated well	5 in each dedicated well
Total Volume	20	20 in each dedicated well	20 in each dedicated well

\*Extra volume of about 10%

5. Mix gently by pipetting up and down.
6. Seal the plate with the optical quality film, without forming any bubbles. If using the QuantStudio™ 5 RT-PCR instrument, use the recommended sealing Foil (Applied Biosystems MicroAmp™ Optical Adhesive Film, at no. 4360954), and if using the CFX96 Touch™ RT-PCR Detection System use the sealing Foil (Bio-Rad Microseal 'B' adhesive seal, cat no. MSB1001).
7. Centrifuge the qPCR plate briefly, to collect reagents at the bottom of the wells.
8. Load the qPCR plate carefully in the qPCR instrument. Even though qPCR actions are stable for up to 20 hours at room temperature, it is recommended to proceed to the qPCR amplification as soon as possible.

### Protocol 3A: qPCR on the QuantStudio™ 5 Real-time PCR System

The following protocol is derived from the Applied Biosystems™ QuantStudio™ 3 and 5 Real-Time PCR Systems – Installation, Use and Maintenance #MAN0010407, revision C.0.

#### Important points before starting

- Take time to familiarize yourself with the QuantStudio™ 5 Real-time PCR system instrument. Read the instrument module user manual.
- The MET™ kit uses the 1.4.2 software version (or higher) of the QuantStudio™ 5 Real-Time PCR System.

#### Things to do before starting

Note: Return kit components to the freezer, to avoid performance degradation.

#### Protocol

1. Turn on the QuantStudio™ 5 Real-Time PCR System instrument.
2. Load the plate into the QuantStudio™ 5 Real-Time PCR System instrument.
3. Start the QuantStudio™ 5 Real-Time PCR System software.
4. Click on “Create New Experiment”

#### Things to do before starting

Note: Return kit components to the freezer, to avoid performance degradation.

#### Protocol

1. Turn on the QuantStudio™ 5 Real-Time PCR System instrument.
2. Load the plate into the QuantStudio™ 5 Real-Time PCR System instrument.
3. Start the QuantStudio™ 5 Real-Time PCR System software.
4. Click on “Create New Experiment”
5. In “Properties” tab,
  - a. Name: enter the name for your experiment.
  - b. Instrument Type: select the cycler to be used “QuantStudio™ 5 Real-time PCR System”.
  - c. Block Type: select the “96-Well 0.2-mL block”.
  - d. Experiment type: select “Comparative Ct ( $\Delta\Delta C_t$ )”.
  - e. Chemistry: select “SYBR Green Reagents”.
6. In “Method” tab,
  - a. Program the QuantStudio™ 5 Real-Time PCR System instrument with the cycling program as indicated in Table 3. Of note, make sure you have selected the “Step and Hold” option for the Melt curve stage.
  - b. Enter a volume of 20  $\mu$ l.
  - c. Click on “Next”.

TABLE 3 QUANTSTUDIO™ 5 REAL-TIME PCR SYSTEM QPCR CYCLING PROGRAM

Cycles	Melt	Temp	Time	Acquisition	Stage
1	N/A	95°C	3 min	None	Initial denaturation
44	N/A	95°C	30 sec	None	PCR stage
	N/A	66°C	30 sec	None	
	N/A	72°C	30 sec	YES	
1	+0.5°C	65°C	5 sec	None	Melt curve stage (Step and Hold)
1		95°C	5 sec	YES	
1	N/A	10°C	10 min	None	Hold Stage

7. In “Plate” tab, enter the sample names and the targets.
  - a. Add 2 targets and rename it as “18S rRNA” and “8.7 kb DEL”.
  - b. Select Reporter “SYBR” and Quencher “None”, Passive Reference is “ROX”.
  - c. Choose one color for each target, in order to be able to distinguish them during analysis.
  - d. Add as many samples as you wish to test and enter the sample names.
  - e. Assign a target and a sample to each well.
  - f. Click on “Next”.

**Important Note:** Please make sure that the “18S rRNA” target is tested in single replicate for each sample and control, and 8.7 kb DEL target is tested in triplicate for each sample and control.

Note: Sample and target can also be imported: file → Import plate set-up. In the “Select file” dialog box, open the corresponding file and click “Select”. After successful file selection, click the “Apply” button. Confirm your choice by clicking on “Yes”. A dialog box will appear to confirm that the import is successful.

8. In the “Run” tab, click the “Start run” button.
9. When the run is finished, select the entire plate, go in the “Results” tab, click on the analysis settings (icon on the right top corner) and select “Relative threshold” in the “Ct settings” tab to obtain Crt values instead of Ct. Press “Apply”. Of note, Crt and Cq are considered equivalent in the MET™ assay and are referred as Cq in this handbook.
10. In the “Export” Tab, select the location of data storage and click on “Export”. An excel file containing the raw data will be automatically generated and stored.
11. Save the report to have the whole summary of your experiment. For this purpose, Click on File – Print report. The report will include all information (experiment summary, thermal protocol, plate layout, results, melt curves...).
12. Unload the QuantStudio™ 5 Real-Time PCR System instrument and discard the plate without removing optical seal according to your local safety regulations.
13. Switch off the qPCR instrument.

## Protocol 3B: qPCR on the CFX96 Touch™ Real-Time PCR Detection System

The following protocol is inspired from the CFX Real-time PCR Detection Systems Instrument Guide #10000068706 Ver A and the CFX Maestro Software – User Guide Version 1.0.

### Important points before starting

- Take time to familiarize yourself with the CFX96 Touch™ Real-Time PCR Detection System before starting the protocol. Read the instrument module user manual.
- The MET™ Real-Time PCR kit design is compatible with the CFX Maestro software version 1.1 (or higher) of the CFX96 Touch™ Real-Time PCR Detection System.

### Things to do before starting

Note: Return kit components to the freezer, to avoid performance degradation.

### Protocol

1. Turn on the CFX96 Real-Time PCR Detection System instrument.
2. Load the plate into the CFX96 Real-Time PCR Detection System instrument.
3. Start the CFX96 Real-Time PCR Detection System software.
4. In the Home Window, click on “File”, then “New > Protocol” to open the Protocol Editor window.
5. In settings, set the following:
  - a. Enter a volume of 20 µl.
  - b. Lid: 105 °C.

6. Set the temperature and time as indicated in the following qPCR program (Table 4).

TABLE 4 CFX96 TOUCH™ REAL-TIME PCR DETECTION SYSTEM qPCR CYCLING PROGRAM

Cycles	Melt	Temp	Time	Acquisition	Stage
1	N/A	95°C	3 min	None	Initial denaturation
43*	N/A	95°C	30 sec	None	Cycling stage
	N/A	66°C	30 sec	None	
	N/A	72°C	30 sec	YES	
1	+0.5°C	65°C	5 sec	None	Melt curve stage
1		95°C	5 sec	YES	
1	N/A	10°C	10 min	None	Hold stage

\*Of note, 44 amplification cycles must be performed. Thus, the 43 repetitions must be programmed (Go to step 2, 43X).

7. Save the qPCR protocol.
  8. In the Home Window, click on "File", then "New > Plate" to open the Plate Editor window.
  9. Fill with the following information in the User preferences:
    - a. Plate size: 96-well.
    - b. Plate type: Clear.
    - c. Scan mode: SYBR® /FAM (channel 1).
    - d. Fluorophore (dye): SYBR.
    - e. In "Data Analysis": Cq determination Mode: Regression.
  10. Assign sample types (NTC, NEC, PC/Calibrator, Unknown) and fluorophore to selected wells.
  11. Assign targets to selected wells (18S rRNA and 8.7 kb DEL).
  12. Assign sample names to selected wells.
  13. Save the Plate layout.
  14. From the Home Window, open the Startup Wizard (Select View > Startup Wizard).

15. Select the qPCR instrument and click "User-defined" as run type.
16. From the Protocol Tab, select the protocol described above and verify that details are correct.
17. From the Plate Tab, select the plate layout.
18. From the Start Run, select the qPCR block that will perform the run.
19. Start the run.

**Important Note:** Please make sure that the 18S rRNA target is tested in single replicate for each sample and control, and 8.7 kb DEL target is tested in triplicate for each sample and control.

Note: Plate and protocols files can also be imported: Open the ExpressLoad folder - Copy plate files (or protocol files) and add them to the dropdown list.

20. When the run is finished, a .pcrd file can be emailed (if an email address has been provided).
  21. Open a Data Analysis window (Home window → File → Open → Data File).
  22. In Setting → Cq determination mode → Regression, go to "Quantification data" and right click to export an excel file containing the "Quantification Cq Results".
  23. In Setting → Cq determination mode - Regression, go to Melt Curve data, right click to export an excel file containing the "Melt curve Peaks Results".
  24. Go to Tools → Reports and select the melt curve option, update the report.
  25. Save the report to have the whole summary of your experiment. For this purpose, click on Tools → Reports. The report will include all information (experiment summary, thermal protocol, plate layout, results, melt curves).
  26. Unload the CFX96 Real-Time PCR Detection System instrument and discard the plate without removing the optical seal, according to your local safety regulations.
  27. Switch off the qPCR instrument.

# Interpretation of results

## Cq description

During qPCR, amplified DNA is fluorescently labelled with the SYBR® Green I dye. The fluorescence intensity is directly proportional to the amount of amplified DNA and monitored during the whole PCR process (along the 44 cycles). The amplification cycle where fluorescence is detected above a baseline is called “quantitation cycle” (Cq). Cq is the basic result of qPCR: when Cq values are lower, more target copies are present in the samples.

Note: Cq and Crt are considered identical and their terminologies depend on the qPCR platform.

## Quality checks and data interpretation

The analysis is done manually, with the following instruction.

Quality checks and data interpretation are divided into 4 steps:

1. Determination of the Assay/Run validity.
2. Determination of the Sample validity.
3. MET™ score calculation.
4. MET™ score adjustment with the PC/Calibrator.

### 1. Determination of the Assay/Run validity

To determine the assay validity, run controls (PC/calibrator (Positive control/ Calibrator), NTC (No Template Control) and NEC (Negative Extraction Control)) are analyzed. The validity of each control is based on Cq and Tm compliance to pre-defined specifications (see Table 5) and interpreted as follow (and in Table 7):

- A control (PC/Calibrator, NTC and NEC) fails if:
  - The 18S rRNA sample is out of the specifications, or
  - Two 8.7 kb DEL replicates out of three are out of the specifications.
- If the PC/calibrator or the NTC fails to meet the Assay/Run validity criteria, the qPCR must be repeated, after investigating the cause of failure.
- If the NEC fails to meet the Assay/Run validity criteria, the DNA extraction and the qPCR must be repeated, after investigating the cause of failure.
- If every control meets the Assay/Run validity criteria, the assay is validated, and analysis can be pursued.

TABLE 5: ASSAY/RUN AND SAMPLE VALIDITY CRITERIA

Target	PC/Calibrator			NTC		NEC	
	Tm range	Cq* range	Cq* reproducibility	Tm range	Cq* range	Tm range	Cq* range
18S rRNA	[80,0°C – 83,0°C]	[26,00; 29,00]	N/A	∇ Tm	>33,00	∇ Tm	>33,00
				∉ [80,0 ; 83,0]	≤33,00	∉ [80,0 ; 83,0]	≤33,00
8.7 kb DEL	[78,0°C- 80,0°C]	[29,00; 32,00]	≤1.50	∇ Tm	>37,94	∇ Tm	>37,94
				∉ [78,0 ; 80,0]	≤37,94	∉ [78,0 ; 80,0]	≤37,94

Note: Cq and Crt are considered identical and their terminologies depend on the qPCR platform.

If the above criteria are met, an additional criterium concerning the CALfactor (difference between the Cal assigned value and the Cal MET™ score of a run) is applied (see Table 6). This criterium validates a qPCR run if the CALfactor is below a defined “acceptable difference”. The acceptable difference was fixed to ±1,44 MET score.

TABLE 6 – VALIDITY CRITERIA FOR THE CAL FACTOR

ID	Target	Replicate	condition	Quality check
CAL	CALfactor (ΔMET score)	1	$ \text{MET}_{\text{assigned}} - \text{MET}_{\text{Calibrator}}  \leq 1,44$	OK

### 2. Determination of the sample validity

The validity of each sample is based on Cq and Tm compliance to pre-defined specifications (see Table 7) and interpreted as follow (see Table 8):

- A sample fails if:
  - The 18S rRNA target is out of the specifications, or
  - Two 8.7 kb DEL replicates out of three are out of the specifications.
- If the sample fails to meet the Sample validity criteria, the qPCR must be repeated, after investigating the cause of failure.
- If the sample meets the Sample validity criteria, the sample is validated, and analysis can be pursued.

TABLE 7 SAMPLE VALIDITY CRITERIA

Target	Sample		
	Tm range	Cq* range	Cq* reproducibility
18S rRNA	[80,0 °C – 83,0 °C]	[22,00; 30,00]	N/A
8.7 kb DEL	[78,0 °C- 80,0 °C]	<37,94	≤1.50
	∇Tm	Undetermined (≥37,94)	≤1.50

\*Cq and Crt are considered identical and their terminologies depend on the qPCR platform.

TABLE 8 RESULTS AND ACTIONS

Sample interpretation	Sample type	Description	Action
Valid	PC/calibrator, NTC, NEC, Test sample	Control or test sample is valid	Proceed with analysis
Invalid	NEC	Extraction Control is invalid	Repeat the entire extraction and qPCR run
Invalid	PC/Calibrator, NTC	qPCR Control is invalid	Repeat the entire qPCR run
Valid	CAL <sub>factor</sub>	The PC/Calibrator results gave the expected score	Proceed with analysis
Invalid	CAL <sub>factor</sub>	qPCR is invalid	Repeat the entire qPCR run
Invalid	Test sample	Test sample is invalid	Launch a new run using the invalidated sample

### 3. MET™ score calculation

A MET™ score is defined as:

$$\text{MET}^{\text{TM}} = \text{Median Cq}_{8.7 \text{ kb DEL}} - \text{Cq}_{18\text{S rRNA}}$$

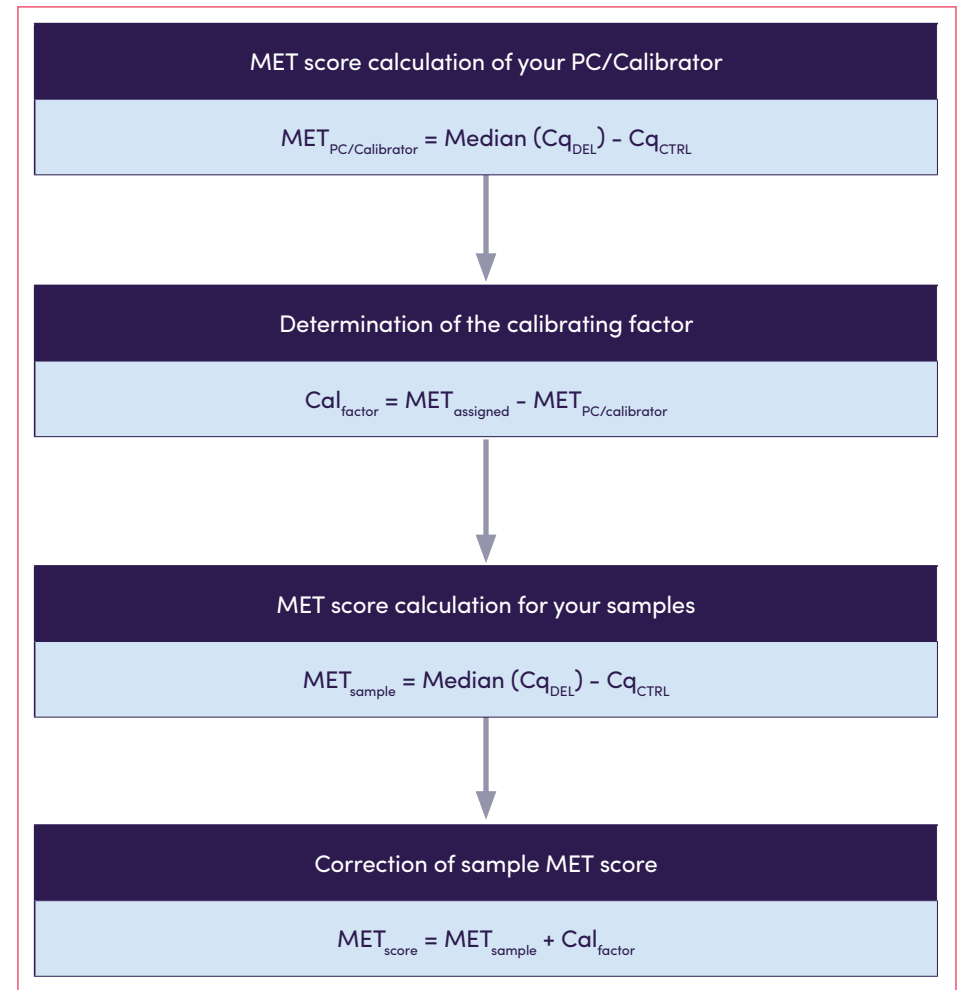
### 4. MET™ score adjustment with the PC/Calibrator

A PC/Calibrator assigned value is reported in its associated CoA and is unique for each lot. It can be found on the internet at [www.mdnalifesciences.com/mitomic-tests](http://www.mdnalifesciences.com/mitomic-tests). The calibration can be done manually.

The Calibration factor is calculated as follow, and described in the figure 6:

1.  $\text{CAL}_{\text{factor}} = \text{MET}_{\text{PC/Calibrator assigned value}} - \text{MET}_{\text{PC/calibrator of your experiment}}$
2.  $\text{MET}^{\text{TM}} \text{ score} = \text{MET}_{\text{sample}} + \text{CAL}_{\text{factor}}$

FIGURE 6 MET™ SCORE CALCULATION



## Troubleshooting Guide

Observation	Suggestion
No signal in PC/Calibrator well(s)	
Wrong fluorescence channel	Select the fluorescence channel SYBR.
Wrong qPCR program	Verify the qPCR program (Table 3 for the QuantStudio™ 5 Real-Time PCR System and Table 4 for the CFX96 Touch™ Real-Time PCR Detection System).
Incorrect configuration of the qPCR plate	Verify your plate layout and repeat the qPCR, if necessary. See Protocol 2: PCR assay set-up, Figures 3 and 4.
Performance degradation	Check the storage conditions and the expiration date of the kit (see the kit label) and use a new kit, if necessary.
Signal in No Template Control well(s)	
Contamination occurred during qPCR	Designate a work area dedicated to PCR assay setup. Decontaminate workspaces and instruments at regular intervals. Follow good laboratory practices. Repeat the qPCR assay with new reagents. Pipet the calibrator last.
Signal in Negative Extraction Control well(s)	
Contamination occurred during extraction	Designate a work area dedicated to DNA extraction. Aliquot DNA extraction reagents. Decontaminate workspaces and instruments at regular intervals. Follow good laboratory practices.

## Quality Control

In accordance with our Quality Management System, each lot of Mitomic® Endometriosis Test Real-Time PCR kit is tested against predetermined specifications, to ensure consistent product quality.

## Limitations

The Mitomic® Endometriosis Test Real-Time PCR kit – RUO must be used by laboratory professionals, such as technicians and physicians, trained in molecular biology techniques and on the different instruments and associated software, used with the assay.

Strict compliance with the “Information for use” is required for accurate results.

Attention should be paid to expiration dates printed on boxes and labels of every kit components. Do not use expired components.

## References







1. Zondervan KT et al. (2018) Endometriosis. Nature Reviews Disease Primers 4: Article number 9. doi:10.1038/s41572-018-0008-5.
2. Gupta et al. (2016) Endometriosis biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database of Systematic Review 4: Article number CD012165. Doi:10.1002/14651858.CD012165.
3. Harbottle A. et al. (2019) A novel 8.7-kb mitochondrial genome deletion accurately detects endometriosis in the plasma of symptomatic women. Biomarker in Medicine. Doi:10.2217/bmm-2019-0451.
4. Creed J. et al. (2019) Mitochondrial DNA deletions accurately detect endometriosis in symptomatic females of child-bearing age. Biomarker in Medicine. Doi:10.2217/bmm-2018-0419.

## Glossary

∇	For all
CALfactor	Calibration factor
CoA	Certificate of analysis
Cq	Cycle Quantification
CTRL	Control, 18S rRNA
DNA	Deoxyribonucleic acid
DEL	8.7 kb mitochondrial deletion
dNTP	Deoxynucleotide triphosphate
∉	Not in
EDTA	Ethylenediaminetetraacetic acid
ID	Identity
MET	Mitomic® Endometriosis Test
ND2	Nicotinamide adenine dinucleotide (NADH) dehydrogenase 2
NEC	No extraction control
NTC	No template control
PC	Positive control
PCR	Polymerase chain reaction
PSIS	Product Safety Information Sheet
qPCR	Real-time polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
rxn	Reaction
Tm	Melt temperature

## Symbols

The following symbols may appear on the packaging and labeling:

Symbol	Symbol definition
	Use by
<b>REF</b>	Catalog number
<b>LOT</b>	Lot number
	Temperature limitation
	Keep away from sunlight
	Manufacturer
	Consult instructions for use
	Caution
<b>CE</b>	CE mark
<b>EC REP</b>	Authorized representative within EU

## Contact Information

### Technical Support

For technical assistance and more information, please contact [techsupport@mdnalifesciences.com](mailto:techsupport@mdnalifesciences.com)

### Customer Support

Contact the local distributor or visit [www.mdnalifesciences.com](http://www.mdnalifesciences.com)

## Ordering Information

Product	Contents	Cat. no.
Mitomic® Endometriosis Test Real-Time PCR Kit		
Mitomic® Endometriosis Test Real-Time PCR Kit	For 96 reactions	200-01

## Website

[www.mdnalifesciences.com/mitomic-tests](http://www.mdnalifesciences.com/mitomic-tests)

## License and liabilities

[www.mdnalifesciences.com/mitomic-tests](http://www.mdnalifesciences.com/mitomic-tests)

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(Bio-Rad Laboratories, Inc.), DNA™ AWAY (Fisher Scientific),  
Vacutainer® (BD Life Sciences)



**MDNA Life Sciences**

2054 Vista Parkway, Suite 400, West Palm Beach Florida, 33411 USA



HalioDx Luminy Biotech Entreprises, 163 Avenue de Luminy, 13288 Marseille Cedex 9 FRANCE