Oct 31, 2025

Digital PCR Analysis for Mitochondrial DNA Integrity and Copy Number (Absolute Q MAP16 Format)

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gpm1w5gzp/v1

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Protocol Citation: Jenny Ghelfi, Soraya Hezzaz, Sylvie Delcambre, Anne Grünewald 2025. Digital PCR Analysis for Mitochondrial DNA Integrity and Copy Number (Absolute Q MAP16 Format). **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gpm1w5gzp/v1

Protocol status: Working
We use this protocol and it's working

Created: October 29, 2025

Last Modified: November 01, 2025

Protocol Integer ID: 231061

Keywords: dPCR, mitochondrial deletion, mitochondrial transcription, copy number, digital pcr analysis for mitochondrial dna integrity, digital pcr analysis, mitochondrial dna integrity, digital pcr, mitochondrial dna, setup of digital pcr, mtdna

Funders Acknowledgements:
Michael J. Fox Foundation
Grant ID: 15744
Michael J. Fox Foundation
Grant ID: 025802
Michael J. Fox Foundation
Grant ID: 021147

Abstract

This protocol describes the preparation and setup of digital PCR assays to quantify mitochondrial DNA (mtDNA) integrity, deletion load, and copy number using the Absolute Q MAP16 format.

Materials

	A	B	C	D
	Item	Supplier	Catalog / Article No.	Notes
	QuantStudio™ Absolute Q™ MAP16 Plate Kit and Master Mix	Thermo Fisher Scientific	Cat. #A53301	Includes MAP16 plates and 5× Master Mix.
	DLOOP-JUN/QSY Probe	Thermo Fisher Scientific	Custom, Article No. CCU002NR	Target: D-Loop region.
	ND4-ABY/QSY Probe	Thermo Fisher Scientific	Custom, Article No. CCU002NR	Target: ND4 gene.
	B2M-FAM/MGB Probe	Thermo Fisher Scientific	Assay ID APCFGXE, Cat. #43320	Target: B2M (nuclear reference).
	ND1-VIC/MGB Probe	Thermo Fisher Scientific	Assay ID APAANDG, Cat. #44485	Target: ND1 gene.
	Water, nuclease-free	Thermo Fisher Scientific	Cat. #R0582	For all dilutions and reactions.
	TE Buffer, 1× (100 mL)	Promega	Cat. #V6231	Used for probe dilution.
	Ethanol (70%)	VWR International (Avantor)	930.031.006	For surface cleaning.

	A	B	C	D
	Equipment	Brand	Model / SKU	Link / Notes
	QuantStudio™ Absolute Q™ Digital PCR System	Thermo Fisher Scientific	—	Used for MAP16 plate runs.
	Mini Microcentrifuge	Corning	Model 6770	Corning LSE Mini Microcentrifuge
	Vortex Mixer	Thermo Scientific	Cat. #88882011	Basic Vortex Mixer

Protocol materials

70% ethanol **VWR International (Avantor)**

Absolute Q™ DNA Digital PCR Master Mix (5X) **Thermo Fisher Scientific Catalog #**A52490

Water, nuclease-free **Thermo Fisher Catalog #**R0582

Safety warnings

- Wear appropriate personal protective equipment (PPE): laboratory coat, gloves, and safety glasses at all times.
- Clean all work surfaces and equipment with 70% ethanol before and after use.
- Dispose of biological waste, tips, and consumables according to your institution's biosafety and chemical waste disposal procedures.
- Follow the manufacturer's safety data sheets (SDS) for all reagents and probes.

Before start

Samples should be extracted using a clean, preferably column-based method to minimize imaging artifacts during digital PCR. It is recommended to test a small range of concentrations before scaling up to a full plate.

Probe Preparation

10m

1 **Check the format of probes:**

- Use 20× probes directly
- If supplied as 60×, dilute to 20× in 1× TE buffer (20 µL probe + 40 µL TE)

Quencher guideline:
A maximum of two MGB-quenched assays should be combined per reaction to ensure optimal fluorescence separation. QSY quenchers are compatible without limitation.

Digital PCR Setup (Absolute Q MAP16 Format)

40m

2 **Plate Preparation**

2.1 **DNA template:** Use DNA from CSF exosomes, PBMCs, or whole blood

- Avoid impure extracts to prevent imaging artifacts

2.2 **MAP16 plate capacity:**
Up to 16 samples
Unused columns may be sealed and stored for future runs

2.3 **Prepare the master mix for each reaction:**

- 2 µL
  Absolute Q™ DNA Digital PCR Master Mix (5X) **Thermo Fisher Scientific Catalog #**A52490
  , vortex well before pipetting
- 0.5 µL of each 20× probe mix (or 0.166 µL if using 60× probes)
- Add Water, nuclease-free **Thermo Fisher Catalog #**R0582 , variable volume (depending on DNA input later, final volume of master mix and DNA = 10 µL)
- Prepare one extra reaction to account for pipetting loss
- Vortex

2.4 **Combine DNA and master mix:**

- For CSF exosomes: 5 µL DNA + 5 µL master mix
- For PBMC or whole blood DNA: 1.1 µL µL DNA + 8.9 µL master mix

2.5 Vortex and spin down to collect contents

2.6 **Load the MAP16 plate:**

- Add 9 µL sample (DNA and master mix) to each well
- Add 15 µL isolation buffer
- Pipette at a 45° angle to avoid damaging the bottom of wells
- Apply gasket strips after each loaded column
- Seal unused columns

Running the Plate on the Absolute Q

1h 20m

3 **Run preparation**

3.1 **Thermal Cycling Parameters**

- Pre-heat: 96 °C for 10 min
- 40 cycles of:
- 96 °C for 5 s
- 60 °C for 30 s

3.2 **Instrument Preparation and Run Start**

- Clean the instrument surface with 70% ethanol **VWR International (Avantor)** and allow to dry completely.
- Load the prepared MAP16 plate into the Absolute Q instrument
- Verify plate orientation matches the software layout
- Start the run

Expected Results and Data Analysis

20m

4 **Expected Results and Analysis**

4.1 **Expected Results**

- Clean partition images
- Clean separation of positive and negative partitions
- Multiplex fluorophores:
- VIC (ND1), FAM (B2M), ABY (ND4), JUN (D-loop).

4.2 **Analysis**

20m

Quantitative results should be analyzed using the Absolute Q software or equivalent digital PCR analysis platform.
Calculate the following ratios for interpretation of mitochondrial DNA status:

- ND4 / ND1 → indicator of *mtDNA deletion load*
- D-Loop / ND1 → indicator of *mtDNA integrity and replication*
- ND1 / B2M → indicator of *mtDNA copy number per cell*