Data Sheet

Track Cell Health

With NovaQUANT™ Mitochondrial qPCR Panels

Simple qPCR: validated mitochondrial panels with optimized primer pairs

Primary research into cell health requires a targeted approach to quantify key mechanisms behind changes in mitochondrial and cellular conditions.

These new NovaQUANT™ mitochondrial gene panels are some of the first standardized experimental tools for mitochondrial research. The panels enable sensitive, specific real-time quantitative PCR (qPCR) detection of mitochondrial and nuclear DNA, as well as expression profiling of key transcriptionally regulated genes associated with mitochondrial biogenesis and oxidative stress.

NovaQUANT™ assays can enhance your studies of metabolism, cell stress, cardiovascular disease, neurodegeneration, toxicity, cell differentiation, immunology, and much more.

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How NovaQUANT™ Assays Accelerate Your Research

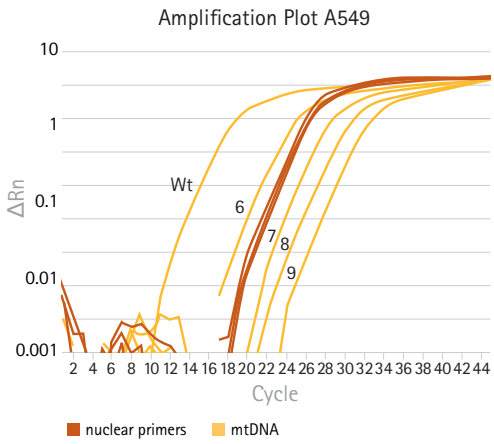
- Validated primer sets ensure accurate quantification
- Verified gene targets from in-house and field publications for both human and mouse samples eliminate time-consuming target validation
- Optimized protocol for sensitive and linear quantification, enabling accurate gene detection
- Pre-aliquoted plate formats with high quality cDNA controls for ultimate convenience

NovaQUANT™ Human and Mouse Mitochondrial to Nuclear DNA Ratio Assays

Accurate determination of the mitochondrial to nuclear DNA ratio is key to assessing cellular homeostasis, which can change with respect to cell differentiation, stress, disease, exercise, caloric intake and toxicity. NovaQUANT™ qPCR assays are an innovative, reliable and user-friendly way to determine the ratio of mitochondrial to nuclear DNA.

Feature		Benefit
Optimized mitochondrial & nuclear primer pairs	=	Matched amplification efficiencies
Validated gene targets	=	Absence of mitochondrial pseudogenes
Optimized protocol for sensitive and linear quantification	=	Accurate gene detection
Validated control (wild type and mitochondrial negative) total DNA	=	High specificity standard(s)
Plates with pre-aliquoted primers	=	Convenient platform

Sensitive and linear detection of key mitochondrial and host cell genes using SYBR® Green Technology



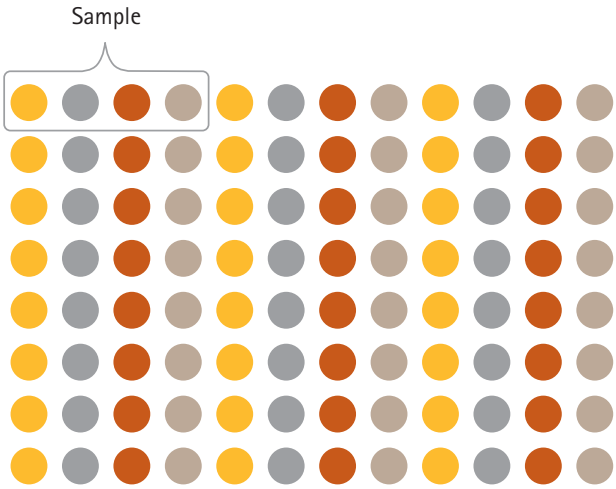
Ethidium Bromide (EtBr)-treated A549 human lung epithelial carcinoma cells were cultured in 50 ng/mL EtBr with passage numbers indicated in black next to curves. Etbr is concentrated differentially in the mitochondria due to higher mitochondrial membrane potential and subsequent DNA binding. Cells were directly lysed in PCR reactions, total DNA normalized to 1 ng/mL and targets amplified using paired mitochondrial (mtDNA) or nuclear primers in a NovaQUANT™ qPCR assay with SYBR® Green technology. Higher passage numbers lead to a greater depletion of mtDNA as cells transition to a glycolytic energy state. Dark lines show no change in nuclear DNA. Wt equals wildtype.

Standardize your protocol for evaluating mitochondrial vs host cell DNA ratios with our human and mouse panels

Human Panel	
Mitochondrial Genes	**Nuclear DNA**
ND1	BECN1
ND6	NEB

Mouse Panel	
Mitochondrial Genes	**Nuclear DNA**
LEV	BECN1
12S	NEB

Plate Layout



ND1
BECN1
ND6
NEB

We recommend SYBR® Green-containing master mix (Toyobo THUNDERBIRD™ (QPS-201) or SA Biosciences Fast SYBR® Mix (PA-042)).

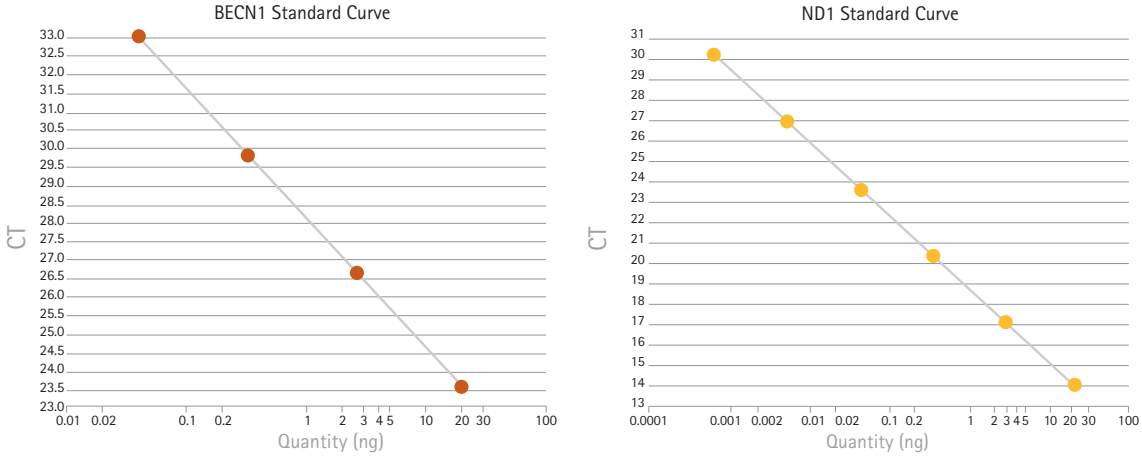
Plate format definition:

4 genes of interest—8 primers total, forward and reverse in each well repeated over 96-well plate

Materials Provided

1. Two qPCR plates with pre-aliquoted PCR primer pairs
2. Wildtype total DNA
3. Mitochondrial minus rho zero DNA

Optimal Sensitivity and Linearity for Technical Needs



Sensitive and Linear Detection of Nuclear (BECN1) and Mitochondrial (ND1) genes. Standard curves run from total DNA isolated from 143B osteosarcoma cells. DNA was run in parallel for the BECN1 nuclear and ND1 mitochondrial gene targets using 8-fold serial dilutions. Both targets show amplification with good linearity, high efficiency and excellent correlation with theoretical amplification as indicated by r2 values of 0.999 for both genes.

Ordering Information

Available from www.merck4biosciences.com

Description	Catalogue No.
NovaQUANT™ Human Mitochondrial to Nuclear DNA Ratio Assay	72620-1KIT
NovaQUANT™ Mouse Mitochondrial to Nuclear DNA Ratio Assay	72621-1KIT

NovaQUANT™ Human and Mouse Mitochondrial Biogenesis and Oxidative Stress qPCR Panels

Mitochondrial biogenesis is activated by cellular stress or in response to external stimuli. As a key regulator of metabolic activity, the mitochondrion produces and degrades free radicals under conditions of oxidative stress.

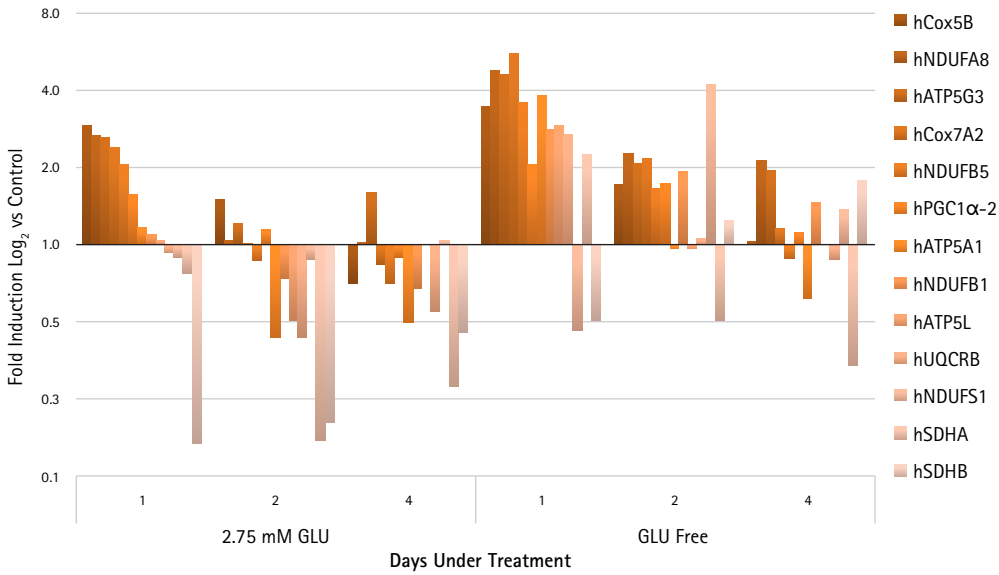
NovaQUANT™ Mitochondrial Biogenesis and Oxidative Stress qPCR Panels are an innovative, reliable and user-friendly way to quantify changes in the expression profiles associated with metabolic and stress pathways.

Features of NovaQUANT™ Mitochondrial Biogenesis and Oxidative Stress Panels

Mitochondrial Biogenesis qPCR Assay Panels	Oxidative Stress qPCR Assay Panels
Panel of 12 biogenesis gene targets tightly controlled by master regulator gene PGC1α	Highly specific panel of 20 transcriptionally regulated oxidative stress genes for unambiguous interpretation

- Stable housekeeping genes offering suitable normalization controls across cell types and experimental conditions for accurate data interpretation and reproducibility
- High quality cDNA for absolute qPCR quantification using standard curves and for assay control
- Pre-aliquoted plate format for convenient set up and data turnaround

Sample Data: Glucose Deprivation Affects Transcription of Mitochondrial Electron Transport Chain Subunit Genes as Detected Using the NovaQUANT™ Human Mitochondrial Biogenesis Panel



To study the effects of glucose starvation on gene expression in the electron transport chain complexes, HepG2 cells were cultured in full-glucose (5.5 mM), low-glucose (2.75 mM) or glucose-free medium. RNA was isolated at days 1, 2 and 4 post-treatment and expression profiling performed using the NovaQUANT™ human mitochondrial biogenesis panel with One-step RNA-direct SYBR® Green Real-time PCR Master Mix (Toyobo) using a StepOnePlus™ Real-Time PCR instrument (Life Technologies). Following normalization to control group (5.5 mM glucose), a marked response in gene expression was shown after 24 hours in both low glucose and glucose free samples, respectively. Subunits from Complex I, IV and V (see panel, next page) were upregulated in the glucose-free and low-glucose samples at 24 hours. Interestingly, both subunits of Complex II (Succinate Dehydrogenase) were down-regulated over all three time points in the low-glucose treatment group. Complex II links oxidative phosphorylation to the Krebs cycle and glucose utilization; therefore, this observation is consistent with a lower glucose level leading to decreased Complex II utilization.

Gene Targets in NovaQUANT™ Mitochondrial Biogenesis Panels

Human Panel		Mouse Panel		
Target	**Accession Number**	**Target**	**Accession Number**	**Function**
hNDUFA8	NM_014222	mNDUFA8	NM_026703	Complex I
hNDUFB1	NM_004545	mNDUFB1	NM_145518	Complex I
hNDUFB5	NM_002492	mNDUFB5	NM_025316	Complex I
hNDUFS1	NM_005006	mNDUFS1	NM_145518	Complex I
hSDHA	NM_004168	mSDHA	NM_023281	Complex II
hSDHB	NM_003000	mSDHB	NM_023374	Complex II
hUQCRB	NM_006294	mUQCRB	NM_026219	Complex III
hCox5B	NM_001862	mCOX5B	NM_009942	Complex IV
hCox7A2	NM_001865	mCOX7A2	NM_009945	Complex IV
hATP5A1	NM_001001937	mATP5A1	NM_007505	Complex V
hATP5G3	NM_001002258	mATP5G3	NM_175015	Complex V
hATP5L	NM_006476	mATP5L	NM_013795	Complex V
hPGC1α-2	NM_013261	mPGC1α-1	NM_008904	Master Regulator
hRPII	NM_000937	mRPII	NM_009089	Housekeeping
hRPL27	NM_000988	mRPL27	NM_011289	Housekeeping
hOAZ1	NM_004152	mOAZ1	NM_008753	Housekeeping

Materials provided for each kit:

1. Two qPCR plates* with pre-aliquoted primer panel
2. Hep G2 cDNA (100 ng at 10 ng/µL or mouse fibroblast (LM) cDNA

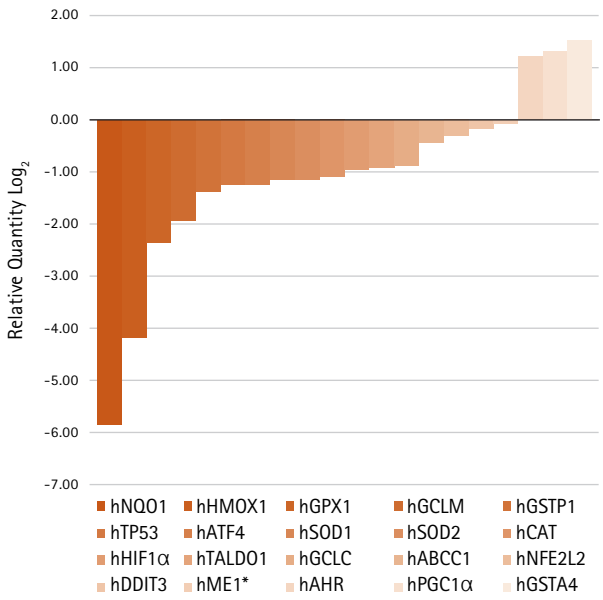
*Applied Biosystems MicroAmp® Fast Optical 96-well Reaction Plate (Cat. No. 4346906), which is compatible with 7500 Fast Real-Time PCR system, 7900HT FAST Real-Time PCR System, and StepOnePlus™ Real-Time PCR Systems.

Ordering Information

Available from www.merck4biosciences.com

Description	Catalogue No.
NovaQUANT™ Human Mitochondrial Biogenesis qPCR Kit	72625-1KIT
NovaQUANT™ Mouse Mitochondrial Biogenesis qPCR Kit	72626-1KIT

Differentiation of hESCs to Hepatocytes Affects Expression of Oxidative Stress Genes as Measured Using the NovaQUANT™ Oxidative Stress qPCR Panel



Human embryonic stem cells (hESCs) were treated with hepatocyte differentiation media. Hepatocyte differentiation was confirmed by down regulation of pluripotency markers as well as functional hallmarks of hepatocyte function such as CYP induction, glycogen storage and albumin production (data not shown). At day 20, RNA was isolated and cDNA reverse transcribed using Merck Millipore's 1st Strand cDNA synthesis kit (Cat. No. 69001). Subsequently, NovaQUANT™ Oxidative Stress qPCR Panel was used to evaluate the stem cell derived hepatocyte oxidative stress signature. The mRNA expression levels shown above were normalized to human cDNA (housekeeping genes) generated from hESCs from which the hepatocytes were derived. The data are presented as fold regulation compared to hESCs on a $\log_2$ scale. Although we anticipated oxidative stress-related genes to be up-regulated due to hepatocytes role in metabolizing toxic compounds, many of the genes were down-regulated compared to hESCs. This may be caused by cellular resources being redirected towards stem cell differentiation.

Gene Targets in NovaQUANT™ Mitochondrial Oxidative Stress Panels

Human Panel		Mouse Panel		
Target	**Accession Number**	**Target**	**Accession Number**	**Function**
hABCC1	NM_004996	mABCC1	NM_008576	Glutathione
hGSTA4	NM_001512	mGSTA4	NM_010357	Glutathione
hGCLM	NM_002061	mGCLM	NM_008129	Glutathione
hGSTP1	NM_000852	mGSTP1	NM_013541	Glutathione
hGCLC	NM_001498	mGCLC	NM_010295	Glutathione
hGPX1(b)	NM_000581	mGPX1	NM_008160	Glutathione
hTALDO1	NM_006755	mTALDO1	NM_011528	Glutathione
hME1	NM_002395	mME1	NM_008615	NADH Process
hNQO1	NM_000903	mNQO1	NM_008706	NADH Process
hSOD1	NM_000454	mSOD1	NM_011434	α-Oxidant Enzyme
hSOD2	NM_000636	mSOD2	NM_013671	α-Oxidant Enzyme
hCAT	NM_001752	mCAT	NM_009804	α-Oxidant Enzyme
hATF4	NM_001675	mATF4	NM_009716	Transcription Factor
hAHR	NM_001621	mAHR	NM_013464	Transcription Factor
hDDIT3	NM_004083	mDDIT3	NM_007837	Transcription Factor
hTP53	NM_000546	mTrp53	NM_011640	Transcription Factor
hNFE2L2	NM_006164	mNFE2L2	NM_010902	Transcription Factor
hHIF1α	NM_001530	mHIF1α	NM_010431	Hypoxia
HMOX1	NM_002133	mHMOX1	NM_010442	Hypoxia
hMT3	NM_005954	mMT3	NM_013603	Hypoxia
hPGC1α	NM_013261	mPGC1α	NM_008904	Master Regulator
hRPII	NM_000937	mRPII	NM_009089	House-keeping
hRPL27	NM_000988	mRPL27	NM_011289	House-keeping
hOAZ1	NM_004152	mOAZ1	NM_008753	House-keeping

Materials Provided for each kit:

1. Two qPCR plates* with pre-aliquoted primer panel
2. HepG2 cDNA or mouse fibroblast LM cDNA (both 100 ng at 10 ng/µL)

* Primers are plated into Applied Biosystems MicroAmp® Fast Optical 96-well Reaction Plate with barcode, 0.1 mL (Cat. No. 4346906), which is compatible with 7500 Fast Real-Time PCR system, 7900HT Fast Real-Time PCR System, and StepOnePlus™ Real-Time PCR Systems.

Ordering Information

Available from www.merck4biosciences.com

Description	Catalogue No.
NovaQUANT™ Human Oxidative Stress qPCR Kit	72627-1Kit
NovaQUANT™ Mouse Oxidative Stress qPCR Kit	72628-1Kit

Other Related Products

More solutions for high-quality PCR. Reliable and robust real-time amplification available from www.merck4biosciences.com

Description	Catalogue No.
NovaTaq™ Hot Start DNA Mastermix	71676

Quantitative PCR of mRNA and crude lysates

Description	Catalogue No.
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FlowCellect™ MitoLive Kit	FCCH100107
FlowCellect™ Annexin Red Kit	FCCH100108
FlowCellect™ MitoStress Kit	FCCH100109
FlowCellect™ Cytochrome c Kit	FCCH100110

Versatility in a Complete Package:

MILLIPLEX® MAP Bead-based Multiplex Assays for Cellular Metabolism available from www.millipore.com

Description	Catalogue No.
MILLIPLEX® MAP Human Fatty Acid Oxidation Magnetic Bead Panel 1	HFA01MAG-11K (magnetic)
MILLIPLEX® MAP Human Fatty Acid Oxidation Magnetic Bead Panel 2	HFA02MAG-11K (magnetic)
MILLIPLEX® MAP Human Oxidative Phosphorylation (OXPHOS) Magnetic Bead Panel	H0XPSMAG-16K (magnetic)
MILLIPLEX® MAP Multi-species Pyruvate Dehydrogenase (PDH) Complex Magnetic Bead Panel (human, mouse, rat)	PDHMAG-13K (magnetic)

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