

Human Mitochondrial DNA Copy Number Assay Kit

(96 well assay suitable for 44 mitochondrial and 44 nuclear reactions, 4 positive controls and 4 blanks)

Catalog Number: MCN 1

Store at -20°C.

FOR RESEARCH USE ONLY



Introduction: This DNA analysis kit is for the determination of human mitochondrial DNA copy number, *in vivo* and *in vitro*, by the comparison of mitochondrial (mt) and nuclear (n) DNA measured by real-time PCR.

Kit Contents:

- 96 well PCR plate
- rtPCR reaction mix.
- Validated primers to quantify mitochondrial DNA (mtDNA).
- Validated primers to quantify nuclear DNA (nDNA)
- Positive control [1.825 ng/ μ l] (isolated human total DNA).

Not Included in Kit:

- DNA isolation Kit
- Nuclease-free water
- PCR Tubes and Caps

Thermal cycler program:

- Preprogram PCR machine for this profile:
 - a. 95°C, 10 min
(40 Cycles)
 - b. 95°C, 15 sec
 - c. 60°C, 60 sec

Real time PCR procedure: The following procedure is for each 20 μ L reaction. Increase all amounts proportionally according to the total number of tubes.

- Per PCR tube (20 μ L Rx), mix the following:
 - a. 1 μ L forward primer
 - b. 1 μ L reverse primer
 - c. 8 μ L sample contain genomic DNA/ 8 μ L of positive control
 - d. 10 μ L rtPCR reaction mix

Recommended concentration: Between 0.5 to 3.75ng/ μ L

Calculations: Mt copy number =

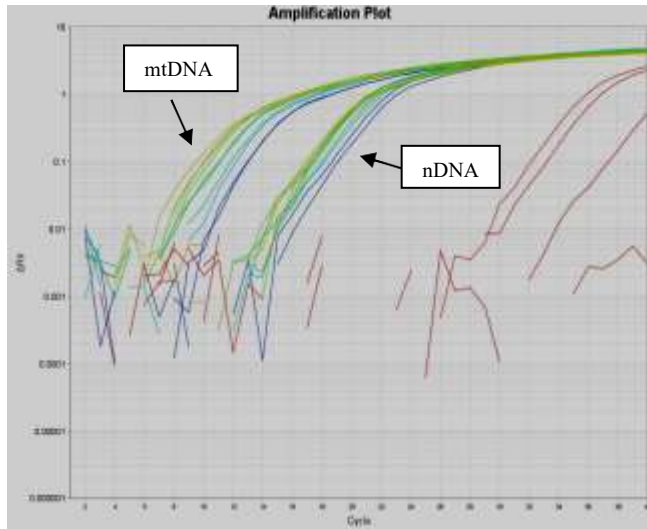
$$\Delta Ct1 = - Ct (\text{mitochondria} - \text{control}) - Ct (\text{nucleus} - \text{control})$$

$\Delta Ct2 = Ct(\text{mitochondria - experimental}) - Ct(\text{nucleus - experimental})$

$\Delta\Delta Ct = \text{Sample } \Delta Ct - \text{Average } \Delta Ct2 \text{ control.}$

mtDNA level change = $2^{-\Delta\Delta Ct}$

Total DNA isolated from human MCF10A cells



- 15.0 ng/uL
- 7.5 ng/uL
- 3.75ng/uL
- 1.825 ng/uL
- 0.9125 ng/uL
- Blank

Suggested assay plate layout: n = nuclear; mt = mitochondria; BLK = blank

	1	2	3	4	5	6	7	8	9	10	11	12
A	nBLK	nS3	nS7	nS11	nS15	nS19	mtBLK	mtS3	mtS7	mtS11	mtS15	mtS19
B	nBLK	nS3	nS7	nS11	nS15	nS19	mtBLK	mtS3	mtS7	mtS11	mtS15	mtS19
C	nPC	nS4	nS8	nS12	nS16	nS20	mtPC	mtS4	mtS8	mtS12	mtS16	mtS20
D	nPC	nS4	nS8	nS12	nS16	nS20	mtPC	mtS4	mtS8	mtS12	mtS16	mtS20
E	nS1	nS5	nS9	nS13	nS17	nS21	mtS1	mtS5	mtS9	mtS13	mtS17	mtS21
F	nS1	nS5	nS9	nS13	nS17	nS21	mtS1	mtS5	mtS9	mtS13	mtS17	mtS21
G	nS2	nS6	nS10	nS14	nS18	nS22	mtS2	mtS6	mtS10	mtS14	mtS18	mtS22
H	nS2	nS6	nS10	nS14	nS18	nS22	mtS2	mtS6	mtS10	mtS14	mtS18	mtS22

Plot of C_T versus DNA concentration

