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/ µl] (isolated total DNA from human MCF10A cells).

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 µL reaction. Increase all amounts proportionally according to the total number of tubes.

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

Recommended concentration: Between 0.5 to 3.75ng/µL

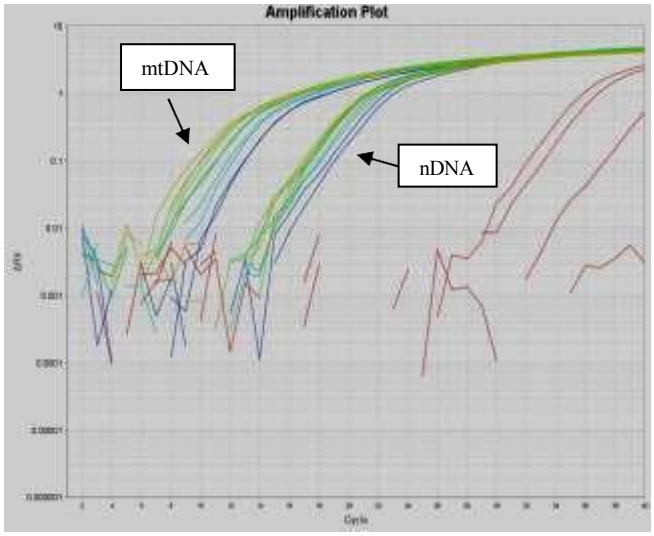
Calculations: Mt copy number =

ΔCt1 =– Ct (mitochondria - control) – Ct (nucleus - control)

2727 Second Ave. Suite 4113 – Detroit, MI 48201 – tel. 313.961.1606 – fax. 313.963.7130 – info@detroitrandd.com – www.detroitRandD.com

ΔCt2 = Ct (mitochondria - experimental) – Ct (nucleus - experimental)
ΔΔ Ct = Sample ΔCt – Average ΔCt2 control.
mtDNA level change = 2^-ΔΔ Ct

Total DNA isolated from human MCF10A cells

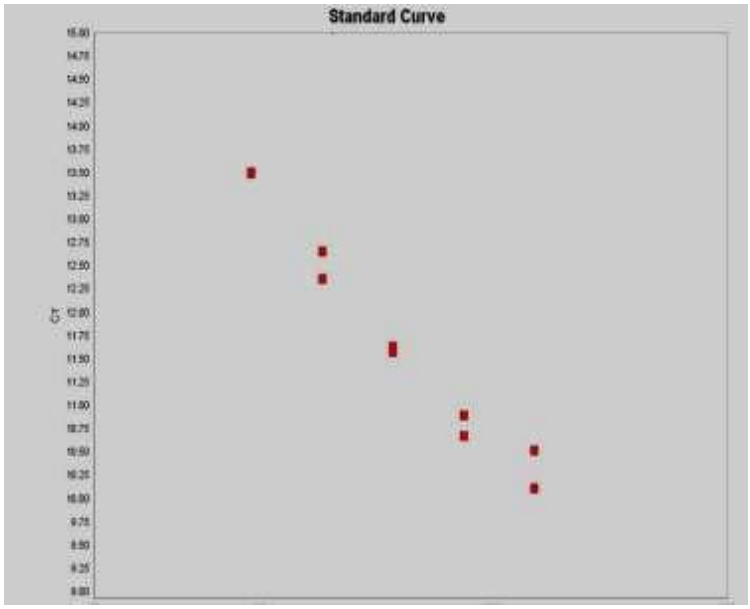


15.0 ng/uL
7.5 ng/uL
3.75ng/uL
1.825 ng/uL
0.9125 ng/uL
Blank

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



References

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