

## Rat Mitochondrial DNA Copy Number

### Assay Kit

(44 reactions)

Catalog Number: MCN 2

Store at -20°C.

FOR RESEARCH USE ONLY



**Introduction:** This DNA analysis kit is for the determination of rat 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/  $\mu$ l] (isolated total DNA from rat NRK-52E 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  $\mu$ L reaction. Increase all amounts proportionally according to the total number of tubes.

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

**Recommended concentration:** Between 0.3 to 5.0 ng/ $\mu$ L

**Calculations: Mt copy number =**

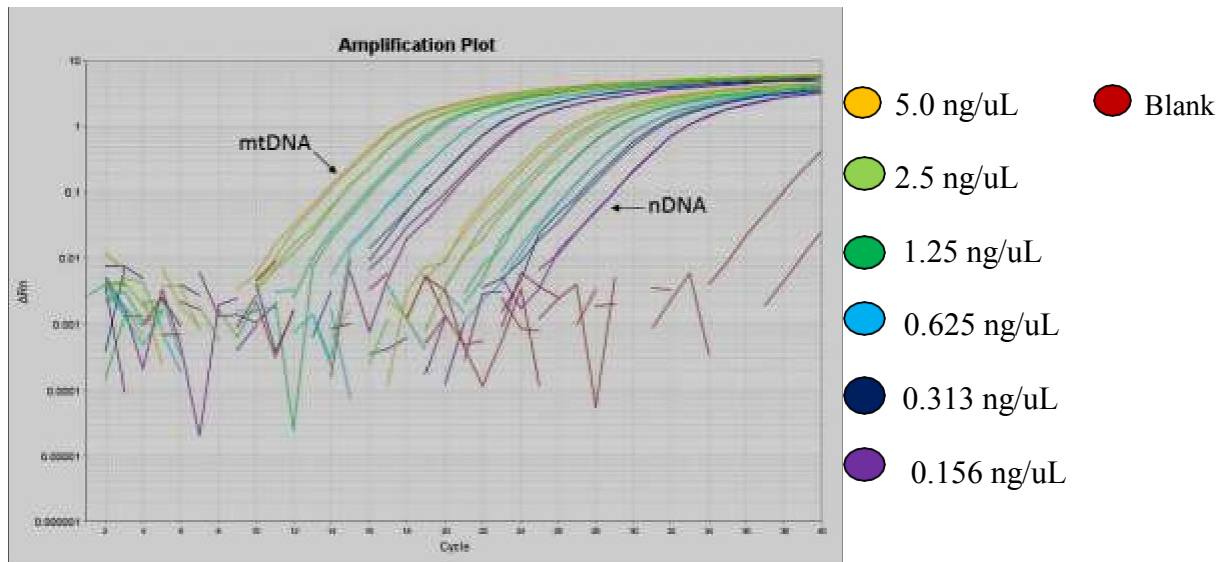
$\Delta Ct1 = Ct (\text{mitochondria -control}) - Ct (\text{nucleus -control})$

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

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

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

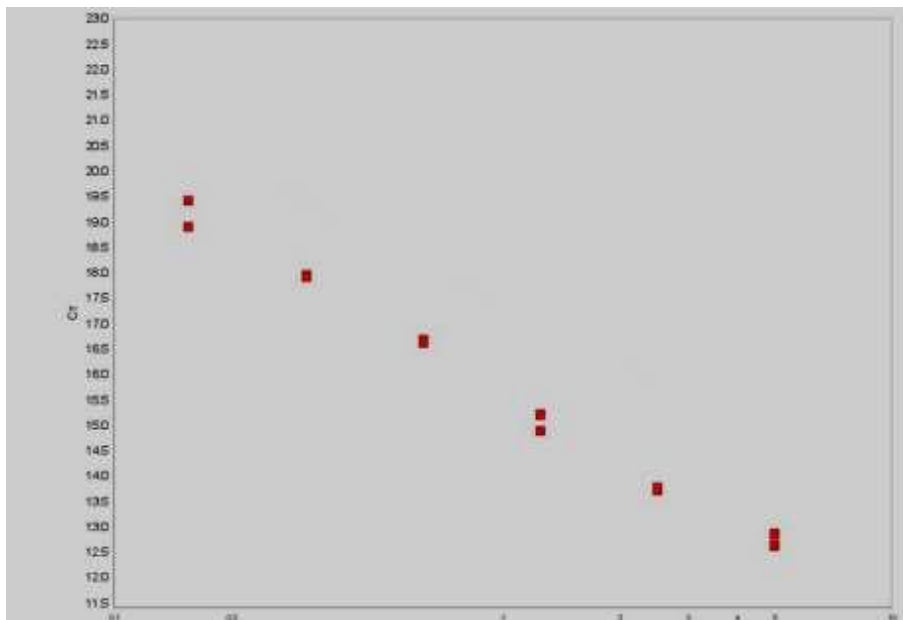
Total DNA isolated from rat NRK-52E cells



Suggested assay plate layout: n = nucleus; 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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2. Dos Santos JM, de Oliveira DS, Moreli ML, Benite-Ribeiro SA. The role of mitochondrial DNA damage at skeletal muscle oxidative stress on the development of type 2 diabetes. *Mol Cell Biochem.* 2018 Dec;449(1-2):251-255. doi: 10.1007/s11010-018-3361-5. PMID: 29679277.  
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3. Mishra JS, Blesson CS, Kumar S. Testosterone Decreases Placental Mitochondrial Content and Cellular Bioenergetics. *Biology (Basel).* 2020 Jul 20;9(7):176. doi: 10.3390/biology9070176. PMID: 32698476.  
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