Human DNA Damage Analysis Kit
Real-Time PCR DNA Quantification following QPCR

(40 reactions)

Catalog Number: DD2H
Store at -20°C.
FOR RESEARCH USE ONLY

Introduction: This DNA damage analysis kit is for the determination of damaged 8.8 kb mitochondrial DNA *in vivo* and *in vitro* in human by quantification of the replicated DNA with real-time PCR following QPCR analysis. This kit allows for duplicate analysis of up to 20 samples (40 reactions).

Contents:

- 2X concentrated QPCR buffer containing polymerase (450 µL)
- QPCR primer mix [2 µM each for forward and reverse primers] (225 µL)
- QPCR test DNA [50 ng/µL] (10 µL)
- 5X Enhancer (180 µL)
- 8.2 kb real-time standard [1 ng/uL] (25 µL)
- Real-time primer mix [5 µM each for forward and reverse primers] (100 µL)

Not Included in Kit:

- SYBR Green Mix (can be purchased separately)
- Nuclease-free water
- PCR Tubes and Caps

1. QPCR thermal cycler procedure

- Preprogram PCR machine for this profile:
    a. 98°C, 30 sec
    b. 98°C, 10 sec
    c. 68°C, 10 sec
    d. 72°C, 4 min
    e. 30 cycles (steps b to d).
    f. 72°C, 10 min
    g. 4°C

Procedure: The following procedure is for each 20 µL reaction. Increase all amounts proportionally according to the total tube number.

- Per PCR tube (20 µL *Rx*), mix the following:
    a. 10.0 µL 2X QPCR concentrated buffer
    b. 4.0 µL 5 X Enhancer
    c. 5.0 µL QPCR primer mix (2 µM each primer, forward/reverse)
    d. 1.0 µL DNA (50 ng/µL)

2. Real-Time PCR procedure (for 20 µL real-time PCR reaction)

- **It is recommended that the PCR DNA product from QPCR be diluted 10-fold with nuclease free water prior to proceeding with the real-time PCR step.**

- Mix the following:
  - 10 µL SYBR green mix (Not included in the kit)
  - 8.1 µL H_2O (nuclease-free)
  - 0.9 µL real-time primer mix (5 µM each primer)
  - 1.0 µL DNA sample (PCR-products from above QPCR)

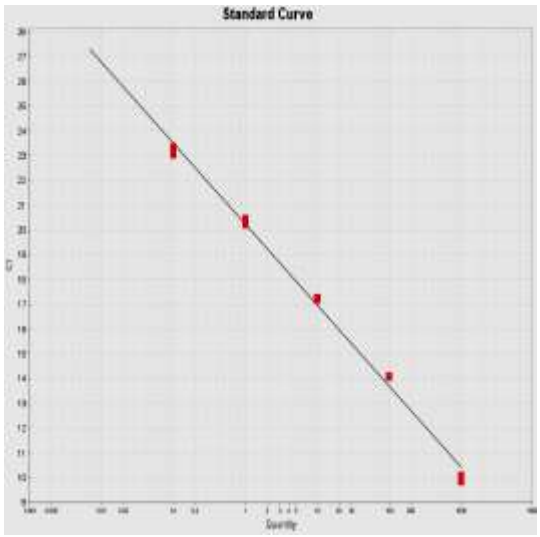
- For the 8.8 kb standard curve, the following optimized DNA concentrations are recommended:
  - 2 ng /2 µL H_2O**
  - 200 pg /2 µL H_2O
  - 20 pg /2 µL H_2O
  - 2 pg /2 µL H_2O
  - 0.2 pg /2 µL H_2O
  - 0.02 pg /2 µL H_2O
  - 0 pg/2 µL H_20

  **8.8 kb real time standard

Recommended Real Time PCR Program

a) 50°C 2 min
b) 95°C 10 min
(program 40 cycles of c and d)
c) 95°C 15 sec
d) 60°C 60 sec

Calculation:
Create a standard curve using the threshold cycle value (C_T) and the DNA concentration (log scale) of each of the 8.8 kb standards. Using linear regression analysis, determine the DNA concentration of your sample based on the C_T value you obtained by PCR . A High level of 8.8 kb product represents less mtDNA damage.


Standard Curve