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

(40 reactions)

Catalog Number: DD2R
Store at -20°C.
FOR RESEARCH USE ONLY

Introduction: This DNA damage analysis kit is for the determination of damaged 8.2 kb mitochondrial DNA *in vivo* and *in vitro* in rat 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 (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. 63°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)

*Vortex stock buffer before aliquoting for reaction

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 folds 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)
  - 7.1 µL H_2O (nuclease-free)
  - 0.9 µL real-time primer mix (5 µM each primer)
  - 2.0 µL DNA sample (PCR-products from above QPCR)

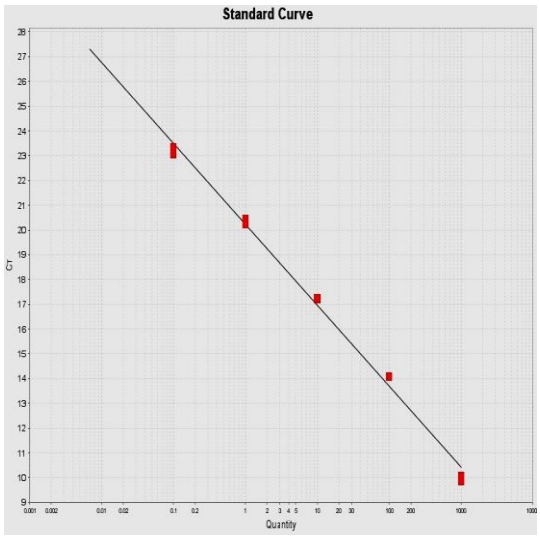
- For the 8.2 kb standard curve, the following optimized DNA concentrations are recommended:
  - 2 ng /2 µL H_2O**
  - 200 pg /2 µL H_2O
  - 20 pg /2 µL H_2O
  - 2 pg /2 µL H_2O
  - 0.2 pg /2 µL H_2O
  - 0.02 pg /2 µL H_2O
  - 0 pg/2 µL H_20

  **8.2 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 8.2 kb standards. Using linear regression analysis, determine the DNA concentration of your sample based on the CT value you obtained by PCR. A High level of 8.2 kb product represents less mtDNA damage.


Standard Curve
CT
Quantity