Detroit R&D, Inc.

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# DNA Damage Analysis Kit

## 8.1-8.8 kb mtDNA target

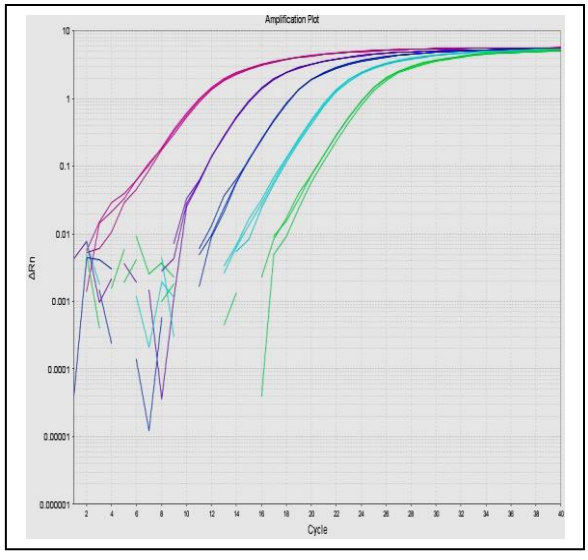
Our DNA damage analysis kits provide a powerful tool for both basic research and industrial applications. This innovative approach is based on performing only 14 to 18 cycles of quantitative PCR on a long (8.8 kb) piece of mitochondrial DNA followed by quantitation using real-time PCR. A critical element is the use of positive and internal controls that allow one to produce a dose-response curve (see below). Choose from human, mouse or rat assay kits.

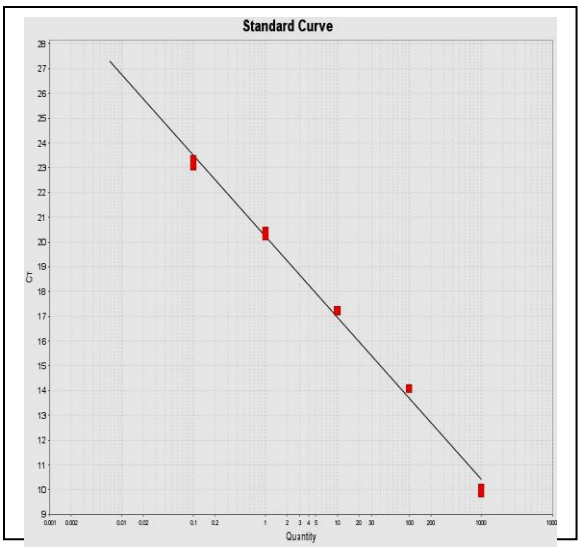
This complete kit can be used for blood, cells or tissue (see below) from human or mouse. DNA damage due to deletions and strand breaks can be detected with this kit. Using a microplate-formatted DNA isolation technology, this kit can be easily used in a high-throughput format. In this respect, the Detroit R&D assay is a promising tool because it is rapid, simple to perform, and requires only a small amount **of test substance**

Cat# DD2H: Real-Time PCR DNA Quantitation following QPCR for human $450/kit
Cat# DD2M: Real-Time PCR DNA Quantitation following QPCR for mouse $450/kit
Cat# DD2R: Real-Time PCR DNA Quantitation following QPCR for rat $450/kit

DNA damage analysis kit for measuring damaged 8.1-8.8 kb mitochondrial DNA **in vivo** and **in vitro** by quantitation of the replicated DNA with real- time PCR following QPCR analysis.

Each kit contains a mitochondrial DNA standard, DNA polymerase, QPCR primers and dNTPs for QPCR and real-time PCR primers, DNA polymerase and an 8.8 kb real-time PCR standard (SYBR Green master mix for real-time PCR is not included) for real-time PCR quantitation. Duplicate analysis of 20 samples (45 reactions).




Left: Amplification Plots for real time PCR standards; Right: Standard curve for real-time PCR analysis of 8.8kb mitochondrial DNA

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# *ENQUIRE!*

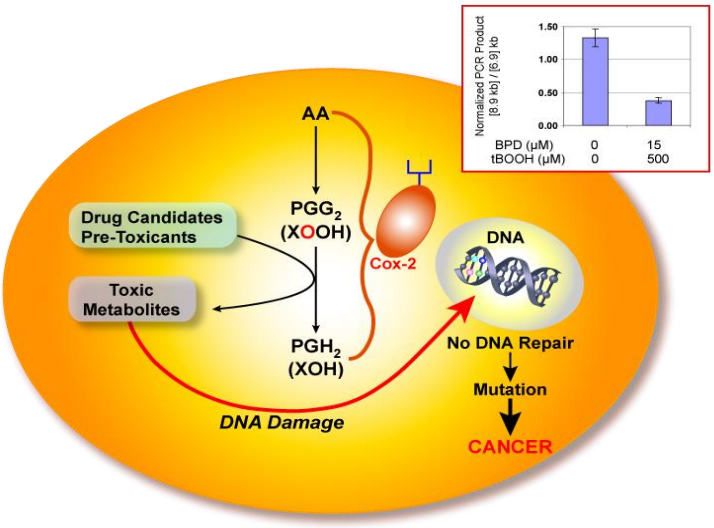
## Catalog # MCN1, MCN2, MCN3 Mitochondrial DNA Copy Number Kits

**A quantitative PCR-based kit for measuring mitochondrial DNA copy number. This kit includes mouse or human or rat primers for an approximately 200 kb mitochondrial DNA sequence. In addition, the kit includes primers for a nuclear DNA (B-actin) sequence, internal control for standard curve, a reaction mixture containing Taq DNA polymerase, dNTPs, MgCl2 and buffer and assay plate.**

# LQPCR Mitochondrial DNA Damage Assay

## using DNA Damage-Sensitive Cox-2-Expressing XPA Cells

### for Pre-Clinical Drug Candidates Screening




**Drug candidates are often biotransformed to reactive, toxic metabolites which damages DNA by peroxidase activity of COX-2. A drug candidate has to be screened at early stage of a drug development for its DNA-damaging activity.**

**Comet and OxyDNA assays detect broken and oxygenated DNAs, respectively. However, thousands of different kinds of DNA adducts formed by drug candidates cannot be detected using the methods. Detroit R&D developed a long quantitative PCR (LQPCR) assay to detect DNA damage induced by thousands of different drug candidates. In the assay, the 8.9 kb mitochondrial DNA fragment is replicated with the 6.9 kb internal control DNA.**

**Detroit R&D developed a COX-2-expressing DNA damage repair-deficient, xeroderma pigmentosum group A (XPA) human fibroblast cell line to sensitize DNA damage occurred by the drug candidates. The COX-2-expressing XPA cells have a robust COX-2 catalytic activity.**

An Example of Utility of LQPCR DNA Damage Assay: **The COX-2-expressing XPA cells were treated with benzo(a)pyrene-7,8-dihydrodiol (BPD), a known COX-2-activated DNA damaging agent, and tert-butyl hydroperoxide (t-BOOH), a substrate of peroxidase activity of COX-2. DNA damage was analyzed by LQPCR. As shown above as a bar graph, COX-2-dependent DNA damage dramatically increased with BPD treatment.**

**In addition to the cell system, the LQPCR DNA damage assay can be used with tissue or blood specimens, fresh or frozen.**

Thus, this LQPCR DNA damage assay can be used to screen drug candidates not only in cell but also in vivo.