DATA SHEET

DNA Damage Quantification Kit

(Catalog #K253-25; 25 assays; Store kit at 4oC, do not freeze)

I. Introduction:

Apurinic/apyrimidinic (AP) sites are one of the major types of DNA lesions formed during the course of base excision and repair of oxidized, deaminated or alkylated bases. It has been estimated that about 2x105 base lesions are generated per cell per day. The level of AP sites in cells can be a good indicator of DNA lesion and repair against chemical damage and cell aging. The *DNA Damage Quantification Kit* utilizes the ARP (Aldehyde Reactive Probe) reagent that reacts specifically with an aldehyde group which is the open ring form of the AP sites. After treating DNA containing AP sites with ARP reagents, AP sites are tagged with biotin residues, which can be quantified using avidin-biotin assay followed by a colorimetric detection. The kit provides the necessary reagents for convenient determination of abasic sites in purified DNA sample in 96-well plate format.

II. Kit Contents:

Component	K253-25 25 assays	Color Code Cap Color	Part Number
ARP Solution (10 mM)	0.125 ml	Red	K253-25-1
TE Buffer	30 ml	NM	K253-25-2
Glycogen Solution (10 µg/µl)	0.1 ml	Blue	K253-25-3
0 ARP-DNA Standard (0.5 µg/ml)	0.6 ml	Clear	K253-25-4
40 ARP-DNA Standard (0.5 µg/ml)	0.6 ml	Yellow	K253-25-5
DNA Binding Solution	10 ml	WM	K253-25-6
HRP-Streptavidin	0.15 ml	Green	K253-25-7
10X Wash Buffer	30 ml	WM	K253-25-8
HRP Developer	10 ml	Brown/NM	K253-25-9
96-well Microplate (8 x 12 strips)	1 plate	Clear	K253-25-10

III. DNA Damage Quantification Protocol:

A. Purification of Genomic DNA:

Several different methods and products are available for isolating genomic DNA. Among all the methods, the guanidine/detergent lysis method is simple, and it gives highly purified genomic DNA for the ARP-based abasic sites detection. During the purification process, avoid heating of the DNA solution. Determine the concentration and purity of the purified genomic DNA using the spectrophotometer*. Dissolve the genomic DNA in TE at concentration of 0.1 µg/µl. It is important for an accurate assay that the DNA concentration is adjusted exactly to 0.1 µg/µl. 1 OD260 nm = 50 µg/ml for genomic DNA. The ratio of OD260 nm/OD280 nm of highly purified DNA solution is 1.7 or higher. Protein contamination in the sample solution may cause a positive error.

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Note: Genomic DNA Isolation Kit is also available from BioVision (Cat.# K281-50). For a positive control, cells may be treated using 10 mM H2O2 for 1 hour at 37C to induce AP site formation.

B. ARP Labeling:
1) Mix 5 µl of 0.1 µg/µl purified sample DNA solution with 5 µl ARP Solution at the bottom of a microcentrifuge tube and incubate at 37oC for 1 hour to tag the DNA AP site.

2) Add 88 µl TE and 2 µl Glycogen to the reaction solution, mix well.

3) Add 0.3 ml of pure ethanol (not provided) mix well and keep at –20oC for 10 min. Centrifuge with microcentrifuge at the top speed for 10 min to precipitate the AP-site tagged DNA.

4) Wash the pellet three times with 0.5 ml 70% ethanol. Quick spin to remove the trace amount of ethanol. Air dry the pellet for 5 min. The Biotin-tagged genomic DNA pellet can be used immediately or store at -20oC. The tagged DNA sample is stable for at least one year.

C. Determination of the number of abasic sites in DNA
1) Dilute the 40 ARP-DNA Standard (40 ARP sites per 105 bp) with 0 APR-DNA Standard to generate 200 µl each of the 0, 8, 16, 24, 32, 40 ARP-DNA solutions in microcentrifuge tubes (see below table).

ARP Number	0	8	16	24	32	40
40 ARP-DNA Standard (µl)	0	40	80	120	160	200
0 ARP-DNA Standard (µl)	200	160	120	80	40	0

2) Dissolve the Biotin-tagged DNA samples prepared in B with 1 ml of TE buffer (0.5 µg/ml).

3) Add 60 µl each of the above ARP-DNA Standards and ARP-labeled DNA samples into each well. For more accurate measurement, use three wells per sample.

4) Add 100 µl of the DNA Binding Solution to the standards and samples, keep the plate at room temperature overnight to allow the tagged-DNA bind on the plate surface. Keep the wells sealed.

Prepare Solutions before use:

Washing Buffer: Dilute the 10X Wash Buffer to 1X Buffer with ddH2O (total volume 300 ml). Store this 1X Wash Buffer at room temperature.

HRP-Streptavidin Solution: Just before use, prepare 1:100 diluted working solution by diluting the 100 µl of HRP-Streptavidin with 9.9 ml 1X Wash Buffer.

5) Discard the DNA Binding Solution in the wells, and wash the well with 250 µl Wash Buffer 5 times.

6) Add 100 µl diluted HRP-Streptavidin solution to each well, and shake the plate for 1 hr at room temperature.

7) Discard the solution in the wells, and wash the wells with 250 µl Wash Buffer 5 times.

8) Add 100 µl of HRP Developer to each well, and incubate at 37oC for 1 hour.

9) Measure the O.D. 650 nm (within 1 hour after the incubation is ended), or add 100 µl of 1 M Sulfuric acid (or 6 M HCl) to stop the reaction. Mix well and measure O.D.450 nm. *

***Note:** The value from O.D.450 nm will roughly double of that from O.D.650 nm.

10) Prepare the calibration curve using the data obtained with standard ARP-DNA solutions. Apply your sample DNA readings to the Standard Curve. The ARP numbers are the abasic sites per 105 bp in the genomic DNA samples. Compare the numbers of AP sites in treated samples vs control samples to determine the level of DNA Damage.