DNA Damage Quantitative Assay Kit

Catalog Number: A21000 (AOXRE Catalog Number 21000)

For Research Use Only. Not For Use in Diagnostic Procedures.

READ ENTIRE INSERT BEFORE BEGINNING ASSAYS!

Store at -20°C.

SUMMARY OF THE TEST

During the course of DNA excision and repair of oxidized, deaminated or alkylated bases, apurinic/apyrimidinic (AP) or abasic sites are formed. It has been estimated that $2x10^5$ base lesions or AP sites are formed per cell per day. The number of AP sites can serve to be an indicator of DNA damage against chemical damage, cell aging and oxidative stress.

PRINCIPLES OF THE PROCEDURE

This kit uses an Aldehyde Reactive Probe (ARP) reagent to react specifically with an aldehyde on the open ring form of AP sites. This allows for the AP sites to be tagged with biotin, which is detected with an HRP-streptavidin conjugate. The color development is directly proportional to the number of AP sites.

MATERIALS PROVIDED

Component	Description	Volume	Storage
ARP Solution	10 mM ARP Solution used to tag the DNA. Red cap.	125 µL	-20°C
Assay Buffer	Tris-EDTA Buffer used for sample preparation.	30 mL	-20°C
Glycogen Solution	10 mg/mL Glycogen Solution used for sample prep. Blue cap.	100 µL	-20°C
Null ARP-DNA Standard	0.5 µg/mL null ARP-site containing DNA standard. Clear cap.	600 µL	-20°C
40 ARP-DNA Standard	0.5 µg/mL 40 ARP per 10^5 bp DNA standard. Yellow cap.	600 µL	-20°C
DNA Binding Buffer	Solution used to coat the plate with sample and standard DNA.	10 mL	-20°C
HRP-Streptavidin	Horseradish peroxidase conjugated streptavidin. Green cap.	100 µL	-20°C
TMB Substrate	Colorimetric substrate for HRP development.	10 mL	-20°C
Multi-Purpose Solution (10X)	Solution used for diluting and washing throughout the assay.	30 mL	-20°C
96-well Microplate	Uncoated clear 96-well microplate.	1 plate	-20°C

MATERIALS NEEDED BUT NOT PROVIDED

1. Microplate reader with a 650 or 450 nm filter
2. Adjustable micropipettes (1 – 1000 µL) and tips
3. Deionized water (DI water)
4. Microcentrifuge tubes
5. Absolute ethanol
6. 1 M Sulfuric acid (H2SO4) (optional)

STORAGE

1. Store the components of this kit at the temperatures specified on the labels.
2. Unopened reagents are stable until the indicated kit expiration date.

WARNING AND PRECAUTIONS

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.

PROCEDURAL NOTES

1. This assay requires an overnight or 12-20 hours incubations time at room temperature. Take note that components should be stored at -20°C at all times when not in use unless otherwise stated.
2. This kit format is intended for use in its entirety of 25 samples in triplicate.
3. To minimize errors in absorbance measurements due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel prior to inserting into the plate reader.

SAMPLE PREPARATION

There are many methods of isolating genomic DNA. It is recommended that the guanidine/detergent method or alternatively the DNeasy Blood and Tissue Kits (Qiagen Cat. No. 69504 for mini spin columns or 69581 for 96-well plates) be used.
Isolated DNA should be diluted with Assay Buffer to a concentration of 0.1 mg/mL. This concentration is essential to the accuracy of the assay.

1. Add 5 µL of 0.1 mg/mL sample to 5 µL of ARP Solution in a microcentrifuge tube, cap and incubate for 1 hour at 37°C.
2. Add 88 µL of Assay Buffer and 2 µL of the Glycogen Solution to each microcentrifuge tube and mix well.
3. Add 300 µL of absolute ethanol to each tube and mix well. Store at –20°C for 10 minutes.
4. Centrifuge the samples at 14,000 x *g* for 10 minutes and discard the resulting supernatant.
5. Wash the pellet three times with 0.5 mL of 70% ethanol. After the final wash briefly centrifuge the tube to collect any residual amounts of ethanol on the walls. Pull off the remaining solution with a pipette and allow most of the ethanol to dry over the course of 3-5 minutes. The resulting Biotintagged DNA pellet can be stored at –20°C for up to one year.

Note: The DNA should not be allowed to dry completely as it will make it difficult to resuspend.

REAGENT PREPARATION

1. **Multi-Purpose Solution (10x):** Add 30 mL of 10x Multi-Purpose Solution to 270 mL of DI water prior to use.
2. **HRP-Streptavidin:** Dilute 1:100 just prior to use. Add 100 µL of HRP-Streptavidin to 9.9 mL Multi-Purpose Solution.

STANDARD CURVE PREPARATION

Use the following table to construct a six-point standard curve.

Table 1: Standard Curve Preparation

Standard	ARP/ 10^5 bp DNA	Vol. of 0 ARPDNA Standard (µL)	Vol. of 40 ARPDNA Standard (µL)	Total Volume (µL)
S1	0	200	0	200
S2	8	160	40	200
S3	16	120	80	200
S4	24	80	120	200
S5	32	40	160	200
S6	40	0	200	200

ASSAY PROCEDURE

1. Dissolve each prepared Biotin-tagged DNA Sample with 1 mL of Assay Buffer to make a final

concentration of 0.5 µg/mL.

2. Place 60 µL of each Sample and Standard to its dedicated wells in triplicate. See Scheme I below for a suggested plate layout.
3. Add 100 µL of DNA Binding Buffer to each well and incubate at room temperature for 12-20 hours.
4. Wash wells 5 times according to the following wash procedure:
   a. Remove the contents of each well by inversion of the plate.
   b. Tap out the remaining contents of the plate onto a lint free paper towel.
   c. Add 250 µL of 1x Multi-Purpose Solution.
   d. Let stand for 2 minutes.
   e. Repeat procedure four more times then proceed to step "f".
   f. Remove the contents of each well by inversion of plate into an appropriate disposal device.
   g. Tap out the remaining contents of the plate onto a lint free paper towel then proceed to step 5.
5. Add 100 µL of freshly diluted HRP-Streptavidin to each well. Allow plate to shake on a plate shaker at 100 revolutions per minute for one hour.
6. Wash wells 5 times according the above wash procedure located in step 4.
7. Add 100 µL of TMB Substrate to each well and incubate for one hour at 37°C.
8. Read plate at 650 nm or stop the reaction with 100 µL of 1 M sulfuric acid and read at 450 nm.

Scheme I: Sample Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S1	U3	U3	U3	U11	U11	U11	U19	U19	U19
B	S2	S2	S2	U4	U4	U4	U12	U12	U12	U20	U20	U20
C	S3	S3	S3	U5	U5	U5	U13	U13	U13	U21	U21	U21
D	S4	S4	S4	U6	U6	U6	U14	U14	U14	U22	U22	U22
E	S5	S5	S5	U7	U7	U7	U15	U15	U15	U23	U23	U23
F	S6	S6	S6	U8	U8	U8	U16	U16	U16	U24	U24	U24
G	U1	U1	U1	U9	U9	U9	U17	U17	U17	U25	U25	U25
H	U2	U2	U2	U10	U10	U10	U18	U18	U18	BLK	BLK	BLK

CALCULATIONS

1. Average all of the duplicate well absorbance values.
2. Graph the standard curve by plotting the ARP/10^5 bp DNA on the x-axis against the OD on the y-axis. Draw a curve using a linear regression curve-fitting routine.
3. Using the standard curve, the number of ARP/10^5 bp DNA of each sample can be determined by comparing the OD of each sample to the corresponding value on the standard curve.

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