Quantification of damaged base in genomic DNA

DNA Damage Quantification Kit-AP Site Counting-

Product Code: DK02

Oxidative damage to DNA is a result of its interaction with reactive oxygen species (ROS), in particular, the hydroxy radical. Hydroxy radicals, which are produced from superoxide anion and hydrogen peroxide by the Fenton reaction, produce multiple modifications in DNA. Oxidative attacks by hydroxy radicals on the deoxyribose moiety will lead to the release of free bases from DNA, generating strand breaks with various sugar modifications and simple abasic sites (AP sites). In fact, AP sites are one of the major types of DNA damage generated by ROS.

Aldehyde Reactive Probe (ARP; N'-aminooxymethylcarbonylhydrazin-D-biotin) reacts specifically with an aldehyde group present on the open ring form of the AP sites (Fig. 1). This reaction makes it possible to detect DNA modifications that result in the formation of an aldehyde group. After treatment with excess ARP reagent, all of the AP sites on DNA are tagged with a biotin residue. These biotin-tagged AP sites can be quantified using the avidin-biotin assay, followed by colorimetric detection with either peroxidase or alkaline phosphatase conjugated to the avidin. DNA Damage Quantification Kit contains all the necessary solutions for detecting between 1 to 40 AP sites per 1 x 10^5 base pairs.

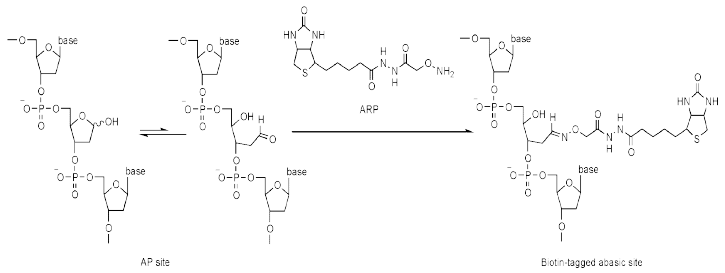

Fig. 1 Reaction of AP site with ARP

1. Purification of genomic DNA

Several different methods and products are available for the isolation of genomic DNA from samples such as membrane binding method, guanidine/detergent lysis method, and polyelectrolyte precipitation method. Among these 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* and agarose gel electrophoresis. Dissolve the genomic DNA in TE at the concentration of 100 µg/ml. It is important for an accurate assay that the DNA concentration is adjusted exactly to 100 µg/ml.

* 1 $OD_{260\,nm}$ = 50 µg/ml. The ratio of $OD_{260\,nm}/OD_{280\,nm}$ of highly purified DNA solution is 1.8 or higher. Protein contamination in the sample solution may cause a positive error.

2. General Protocol

► ARP reaction

1. Mix 10 µl of purified genomic DNA solution(100 µg/ml) and 10 µl of ARP Solution in a 0.5 ml tube, and incubate at 37°C for 1 hour.
2. Wash the inside of the Filtration Tube with 100 µl of TE twice.
3. Add 380 µl of TE to the reaction solution, and transfer the solution to the Filtration Tube.
4. Centrifuge the Filtration Tube at 2,500 xg for 15 minutes, and discard the filtrate solution.
5. Add 400 µl of TE to the Filtration Tube and resuspend the DNA on the filter with a pipette.
6. Centrifuge the Filtration Tube at 2,500 xg for 15 minutes.[a)]
7. Add 200 µl of TE to the Filtration Tube to resuspend the DNA on the filter with a pipette.
8. Transfer the DNA solution to the 1.5 ml tube, and add 200 µl of TE again to the Filtration Tube to transfer the ARP-labeled DNA on the filter completely to the 1.5 ml tube.[b)]
9. Store the ARP-labeled genomic DNA solution at 0 to 5°C.

a) If the DNA solution still remains on the filter after the centrifugation, spin for another 5 minutes.

b) Recovery rate of DNA using the filtration tube is 90%, so the concentration of the ARP-labeled DNA is 2.25 µg/ml. For more accurate determination of the number of abasic sites in the sample DNA, we recommend measuring the DNA concentration.

Contents of the Kit

5 samples

ARP Solution (10mM ARP)	100 µl x 1
ARP-DNA Standard Soln.*	250 µl ea.
(0, 2.5, 5, 10, 20, 40 AP sites/100,000 bp)	
DNA Binding Solution	10 ml x 1
Substrate Solution	10 ml x 1
TE Buffer	15 ml x 1
HRP-Streptavidin	25 µl x 1
Washing Buffer	1 pack
Filtration Tube	5 tubes
96-well Microplate/ U bottom	1 plate

20 samples

ARP Solution (10mM ARP)	250 µl x 1
ARP-DNA Standard Soln.*	250 µl ea.
(0, 2.5, 5, 10, 20, 40 AP sites/100,000 bp)	
DNA Binding Solution	10 ml x 1
Substrate Solution	10 ml x 1
TE Buffer	40 ml x 1
HRP-Streptavidin	25 µl x 1
Washing Buffer	1 pack
Filtration Tube	20 tubes
96-well Microplate/ U bottom	1 plate

Required Equipment & Materials

Microplate Reader (650 nm filter)
10 µl, 100-200 µl and 1 ml pipettes
50-250 µl multi-channel pipettes
Incubator(37°C)
0.5 ml and 1.5 ml tube
Centrifuge
Paper Towel

► Determination of the number of AP site in DNA

Day 1

1. Dilute 90 µl of the ARP-labeled genomic DNA with 310 µl of TE.
2. Add 60 µl of ARP-DNA Standard Solution per well. Use three wells per 1 standard solution.
3. Add 60 µl of the diluted ARP-labeled genomic DNA solution per well. Use at least three wells per 1 sample.
4. Add 100 µl of the DNA Binding Solution to each well, then allow the plate to remain at room temperature overnight.

Day 2

1. Prepare stock solutions
   - » Washing Buffer: Dissolve the contents of the Washing Buffer packet in 1 L of deionized or distilled water. Store this Washing Buffer solution at room temperature.
   - » HRP-Streptavidin solution: Dilute HRP-Streptavidin with Washing Buffer to prepare 1/4000 diluted working solution.*
   - » 1/4000 dilution preparation: Centrifuge HRP-Streptavidin tube for 30 seconds. Add 10 µl of HRP-Streptavidin into 40 ml of Washing Buffer solution, and mix well.

   * Since this working solution is not stable, always use freshly prepared solution.
2. Discard the DNA Binding Solution in the wells, and wash the well with 250 µl Washing Buffer 5 times.
3. Add 150 µl of diluted HRP-Streptavidin solution to each well, and incubate the plate at 37°C for 1 hour.
4. Discard the solution in the well, and wash the well with 250 µl Washing Buffer 5 times.[b)]
5. Add 100 µl of Substrate Solution to each well, and incubate at 37°C for 1 hour.
6. Measure the O.D. at 650 nm within 1 hour after the incubation is finished, and prepare a calibration curve using the data obtained with ARP-DNA Standard solutions.
7. Determine the number of abasic sites in the genomic DNA using the calibration curve.

3. Notes

1. Please store the kit at 0-5°C. Do not freeze. Store Washing Buffer solution at room temperature.
2. AP-DNA is not stable. Please treat it with ARP and purify with Filtration Tube after the isolation of genomic DNA from a sample.
3. Purified ARP-DNA solution in TE buffer is stable over one year at 0-5°C storage.
4. After the spinning of Filtration Tube for ARP-labeled DNA purification, add 200 µl TE immediately. If the DNA stays in Filtration Tube for more than 30 minutes after the spinning, the DNA recovery ratio may decline.
5. γ-Ray-sterilized tubes may cause DNA binding on the surface of the tube during the mixing of the DNA solution with DNA Binding Solution. If you prefer to mix ARP-DNA solution with DNA Binding Solution in a tube rather than mixing them in a well, please avoid using g-ray-sterilized tubes.
6. If the 650 nm filter is not available for the measurement of O.D. after the color development, transfer 50 µl of the solution in each well to a well of a new plate (not provided). Then, add 50 µl of 1 M sulfuric acid, and measure the O.D. at 450 nm.
7. Remaining solution in a well may cause error, so please remove the solution thoroughly by tapping the plate on a paper towel in each step.

4. References

1. T. Lindahl, *et al*., Rate of Depurination of Native Deoxyribonucleic Acid. *Biochemistry*. 1972;**11**:3610-3618.
2. M. Liuzzi, *et al*., A New Approach to the Study of the Base-excision Repair Pathway Using Methoxyamine. *J Biol Chem*. 1985;**260**:5252-5258.
3. A. Sancar, *et al*., DNA Repair Enzymes. *Annu Rev Biochem*. 1988;**57**:29-67.
4. M. Weinfeld, *et al*., Response of Phage T4 Polynucleotide Kinase Toward Dinucleotides Containing Apurinic Sites: Design of a 32P-postlabeling Assay for Apurinic Sites in DNA. *Biochemistry*. 1990;**29**:1737-1743.
5. B. X. Chen, *et al*., Properties of a Monoclonal Antibody for the Detection of Abasic Sites, a Common DNA Lesion. *Mutat Res*. 1992;**273**:253-261.
6. J. A. Gralnick, *et al*., The YggX Protein of Salmonella enterica Is Involoved in Fe(II) Trafficking and Minimizes the DNA Damage Cause by Hydroxyl Radicals:Residue CYS-7 is Essential for YggX Function. *J Biol Chem*. 2003;**278**:20708-20715.

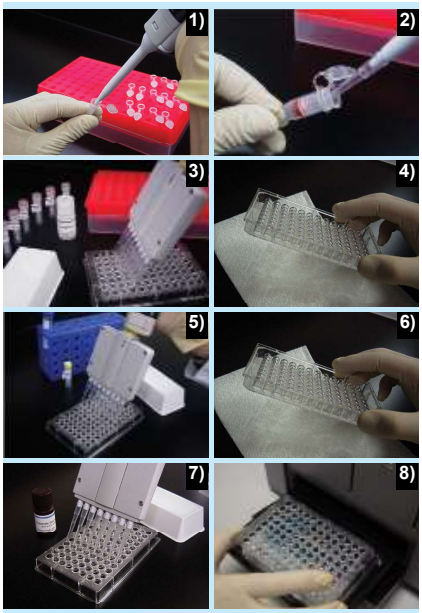

Fig. 2 Assay procedure

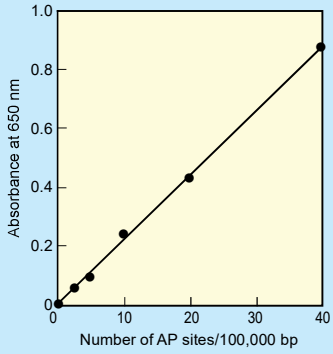

Fig. 3 Typical calibration curve of DNA Damage Quantification Kit

5. Recent Publications

Title	Reference
Novel Role of Base Excision Repair in Mediating Cisplatin Cytotoxicity	A. Kothandapani, and S. M. Patrick, *et al., J. Biol. Chem*. 2011; **286**: 14564 - 14574
Cadmium affects metabolic responses to prolonged anoxia and reoxygenation in eastern oysters (Crassostrea virginica)	I. O. Kurochkin, and I. M. Sokolova, *et al., Am J Physiol Regulatory Integrative Comp Physiol*. 2009; **297**: R1262 - R1272
Impairment of APE1 Function Enhances Cellular Sensitivity to Clinically Relevant Alkylators and Antimetabolites	Daniel R., and D. M. Wilson, *et al., III Mol. Cancer Res*. 2009; **7**: 897 - 906
Temporary Pretreatment With the Angiotensin II Type 1 Receptor Blocker, Valsartan, Prevents Ischemic Brain Damage Through an Increase in Capillary Density	Jian-Mei Li, and M. Horiuchi, *et al., Stroke*. 2008; **39**: 2029 - 2036
Bcl2 Inhibits Abasic Site Repair by Down-regulating APE1 Endonuclease Activity	J. Zhao, and X. Deng, *et al., J. Biol. Chem.* 2008; **283**: 9925 - 9932
Cockayne syndrome B protein stimulates apurinic endonuclease 1 activity and protects against agents that introduce base excision repair intermediates	H. Wong, and D. M. Wilson, *et al., III Nucleic Acids Res.* 2007; **35**: 4103 - 4113
Angiotensin II-Induced Neural Differentiation via Angiotensin II Type 2 (AT2) Receptor-MMS2 Cascade Involving Interaction between AT2 Receptor-Interacting Protein and Src Homology 2 Domain-Containing Protein-Tyrosine Phosphatase 1	Jian-Mei Li, and M. Horiuchi, *et al., Mol. Endocrinol.* 2007; **21**: 499 - 511
A Dominant-Negative Form of the Major Human Abasic Endonuclease Enhances Cellular Sensitivity to Laboratory and Clinical DNA-Damaging Agents	Daniel R. McNeill and David M. Wilson, *III Mol. Cancer Res*. 2007; **5**: 61 - 70
Bcl2 Suppresses DNA Repair by Enhancing c-Myc Transcriptional Activity	Z. Jin, and X. Deng, *et al., J. Biol. Chem.* 2006; **281**: 14446 - 14456
Folate Deficiency Increases Postischemic Brain Injury	M. Endres, and K. Gertz, *et al., Stroke*. 2005; **36**: 321 - 325

6. FAQ

►Can I use single-stranded DNA or RNA?
No, you cannot use this kit to determine the number of abasic sites in single-stranded DNA or RNA. The O.D. reading of single-stranded DNA will be nearly twice that of double-stranded DNA because of the binding efficiency on the microplate.

►How should genomic DNA be stored?
Prepare a DNA pellet and store at -20°C or -80°C if the DNA cannot be labeled with ARP immediately after isolation. After ARP labeling, the sample can be stored at 4°C in TE Buffer for several months.

►How should I prepare the DNA?
You can use general protocols or commercially available DNA isolation kits. Between 2 to 4 abasic sites per 1 x 10^5 base pairs will be created during the DNA isolation process. Therefore, use the same isolation method to prepare each DNA sample.

►How can I determine the number of abasic sites if there are more than 40 per 1 x 10^5 base pairs?
Simply dilute the ARP-labeled sample DNA with 0.5 µg per ml double-stranded genomic DNA, such as calf thymus or salmon sperm DNA, using TE Buffer.

►What should I do if the sample DNA concentration is less than 100 µg per ml?
You can either use a filtration tube to concentrate your sample DNA or ethanol precipitation to recover DNA as a pellet and then re-dissolve it to prepare a 100 µg per ml solution.

►What should I do if the sample DNA is less than 1 µg?
Add the same volume of ARP Solution and follow the manual. The recovery of the ARP-labeled DNA may be lower than the usual reactions, so measure the ARP-labeled DNA solution. The average recovery rate of the 0.5 µg DNA and 0.25 µg DNA is 70% and 50%, respectively.