Product Information

DNA Damage Quantification Colorimetric Kit

I. Kit Contents:

Components	K2100-25 25 assays	Cap Color	Part Number
ARP Solution (10 mM, in DMSO)	0.125 ml	Red	K2100-C-1
TE Buffer	30 ml	NM	K2100-C-2
Glycogen Solution (10 µg/µl)	0.1 ml	Blue	K2100-C-3
0 ARP-DNA Standard (0.5 µg/ml)	0.6 ml	Clear	K2100-C-4
40 ARP-DNA Standard (0.5 µg/ml)	0.6 ml	Yellow	K2100-C-5
DNA Binding Solution	10 ml	WM	K2100-C-6
HRP-Streptavidin	0.1 ml	Green	K2100-C-7
10X Wash Buffer	30 ml	WM	K2100-C-8
HRP Developer	10 ml	Brown/NM	K2100-C-9
96-well Microplate (8 x 12 strips)	1 plate	Clear	K2100-C-10

II. Introduction:

Apurinic/apyrimidinice (AP) sites are a main type of DNA lesions that occurs during base excision and repair of oxidized, deaminated or alkylated bases. The amount of AP sites in cells indicates DNA lesion and repair against cell aging and chemical damage. This kit utilizes the ARP (Aldehyde Reactive Probe) reagent that reacts with aldehyde group of the AP sites. Biotin residues are tagged onto the AP sites following the treatment of DNA containing AP sites with ARP reagents. The biotin-tagged ATP sites can be quantified by avidin-biotin assay and colorimetric detection.

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. 1.0 $OD_{260\ nm}$ = 50 µg/ml for genomic DNA. The ratio of $OD_{260\ nm}/OD_{280\ nm}$ of highly purified DNA solution is 1.7 or higher. Protein contamination in the sample solution may cause a positive error.

Note: Genomic DNA Isolation Kit is also available from BioVision. For a positive control, cells may be treated using 10 mM H_2O_2 for 1 hour at 37℃ 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 37℃ 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 –20℃ 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 -20℃. 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 10^5 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 ddH_2O (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 37℃ for 1 hour.

9) Measure the OD 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 OD 450 nm.

Note: The value from OD 450 nm will be roughly double of that from OD 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 basic sites per 10^5 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.

Frequently Asked Questions

1. When setting up analyses of DNA from genomic DNA samples it states that the concentration should be 0.1 microgram per microlitre. We have some samples that are about 10 times less concentrated and wondered if it was possible to adjust any of the volumes to take this into account?

Yes, it is essential that the DNA start conc is 0.1 µg/µl. We ask you to resuspend your genomic DNA after isolation in that conc. If you have a more dilute version, I would recommend you to concentrate the DNA to the recommended conc, by doing an agarose extraction, or any such approach. Failing this, the protocol will have to be completely modified for the various reagent volumes and you might need to do some optimization steps.

2. Did I understood correctly, that the biotin-labeled-DNA binds to the microplate due to interactions to avidin? Can you tell me what the Binding-Solution contains. Also in the instruction is written, that for the binding to the microplate there is an incubations time over night. Is there the opportunity to shorten this incubation time?

The DNA-ARP-Biotin binds to the plate with the help of the DNA binding solution. This solution does not have any Avidin. I am sorry, but I cannot disclose the components of the DNA binding buffer due to proprietary restrictions. The binding would be most efficient when incubated O/N, I would not recommend shortening this time.

3. If the provided 96-microplate in the kit is a normal once and what are the standards consisting of?

The standards consist of DNA which has already been labeled with ARP, at either 0 or 40 sites/10^5 bps.

4. We have purchased this DNA damage kit and can I get some raw data of standard curve (absorbances of different standards). As I am planning to test it first in my samples (with conc less than 0.1 µg/ul by using more volume than recommended). I wanted to compare if my samples are falling within the range of standards.

It's not a good idea for your to compare your sample readings with our standards. I would recommend you to make your own standards to see if your sample readings fall within its linear range.

5. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

6. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

7. Can we use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

8. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

9. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

10. Why are my standard curve values lower than those shown on the data sheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

11. How do I normalize my samples against protein concentration?

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

12. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

13. Should I make a standard curve for every expt I do, or is one curve/kit enough?

Yes, It is recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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