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ab211154 DNA damage – AP sites – Assay Kit (Colorimetric)

For the rapid and sensitive detection of aldehyde site (AP sites) in purified DNA samples.

This product is for research use only and is not intended for diagnostic use.

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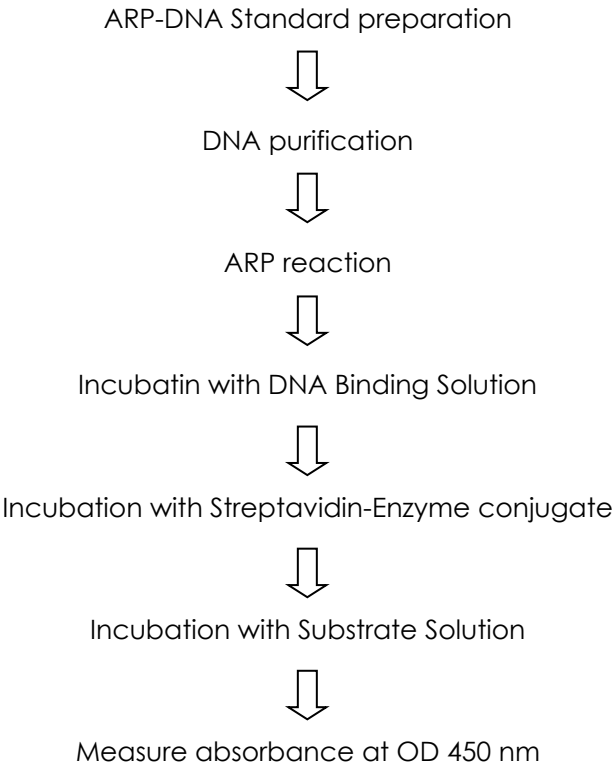
1. Overview

DNA damage – AP sites – Assay Kit (Colorimetric) (ab211154) provides a sensitive and specific method to monitor the formation of apurinic/apyrimidinic (AP) sites, one of the major types of DNA lesions. The assay uses an APR (Aldehyde Reactive Probe) that reacts specifically with an aldehyde group on the open ring form of AP sites. AP sites are then tagged with biotin residues that can later be quantified using an streptavidin-enzyme conjugate that is easily detected by absorbance at OD450 nm. The kit has a detection sensitivity range of 4-40 AP sites per 1 x 10^5 bp.

Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged lipids and proteins. DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers, such as those of the colon, breast, rectum, and prostate. Among numerous types of oxidative DNA damage, apurinic/apyrimidinic (AP or abasic) site is one of the prevalent lesions of oxidative DNA damage. Abasic sites arise in DNA at a significant rate by spontaneous base loss as in depurination, by DNA oxidation, or by the action of DNA glycosylases. Estimates of the number of abasic sites generated per mammalian cell run as high as 50,000 to 200,000 per day. Unrepaired abasic sites inhibit topoisomerases, replication, and transcription and can be mutagenic because of bypass synthesis on nontemplated DNA.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C in the dark (reduced DNA standard and ARP-DNA standard should be stored at -20°C) immediately upon receipt. Kit has a storage time of 1 year from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: avoid multiple freeze/thaw cycles.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity		Storage temp (before prep)	Storage temp (After prep)
	10 tests	50 tests		
10X Wash Buffer	10 mL	30 mL	4°C	4°C
ARP Solution	50 µL	250 µL	4°C	4°C
ARP-DNA Standard	75 µL	400 µL	-20°C	-20°C
DNA Binding Solution	1.25 mL	6 mL	4°C	4°C
DNA High-Binding Plate	1 x 32 wp	1 x 96 wp	4°C	4°C
Glycogen Solution	10 µL	100 µL	4°C	4°C
Reduced DNA Standard	200 µL	1 mL	-20°C	-20°C
Sodium Acetate Solution	200 µL	1 mL	4°C	4°C
Stop Solution	4 mL	12 mL	4°C	4°C
Streptavidin-Enzyme Conjugate	10 µL	20 µL	4°C	4°C
Substrate Solution	4 mL	12 mL	4°C	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450 nm (OD 620 nm can be used as optional reference wavelength)
- Double distilled water (ddH_2O)
- TE Buffer [10 mM Tris pH 7.5, 1 mM EDTA]
- Ethanol: 100% and 70%
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- (Optional) Genomic DNA Isolation Kit (ab65358): to isolate genomic DNA from treated cell or tissue samples

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 10X Wash buffer:

Dilute the 10X Wash Buffer in ddH_2O to create **1X Wash Buffer**. Stir the solution to homogenize. Equilibrate to room temperature before use. Store unused 1X Wash Buffer at 4°C.

9.2 ARP Solution (10 mM):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot so that you have enough to perform the desired number of assays. Store at 4°C.

9.3 ARP-DNA Standard (40 ARP/10^5 bp - 6 µg/mL in TE buffer):

Ready to use as supplied. Aliquot ARP-DNA standard so that you have enough to perform the desired number of assays. Avoid multiple freeze/thaw cycles. Store at -20°C.

9.4 DNA Binding Solution:

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.5 DNA High-binding Plate:

Ready to use as supplied. Equilibrate to room temperature before use. Store unused strips in the pouch provided at 4°C.

9.6 Glycogen Solution (10 mg/mL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot so that you have enough to perform the desired number of assays. Store at 4°C.

9.7 Reduced DNA Standard (6 µg/mL in TE buffer):

Ready to use as supplied. Aliquot reduced DNA standard so that you have enough to perform the desired number of assays. Avoid multiple freeze/thaw cycles. Store at -20°C.

9.8 Sodium Acetate Solution (3 M, pH 5.5):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot so that you have enough to perform the desired number of assays. Store at 4°C.

9.9 Stop Solution:

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot so that you have enough to perform the desired number of assays. Store at 4°C.

9.10 Streptavidin-Enzyme Conjugate:

Ready to use as supplied. Aliquot so that you have enough to perform the desired number of assays. Store at 4°C.

Immediately prior using, dilute Streptavidin-Enzyme Conjugate 1:1000 dilution in 1X Wash Buffer. Do not store diluted conjugate.

9.11 Substrate Solution:

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot so that you have enough to perform the desired number of assays. Store at 4°C.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Using ARP-DNA standard (Step 9.3) and reduced DNA standard (Step 9.7), prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	ARP-DNA Standard (µL)	Reduced DNA Standard (µL)	TE Buffer (µL)	Final volume standard in well (µL)	AP Sites per 10^5 bp
1	20	0	100	50	40
2	16	4	100	50	32
3	12	8	100	50	24
4	8	12	100	50	16
5	4	16	100	50	8
6	2	18	100	50	4
7	1	19	100	50	2
8 (blank)	0	20	100	50	0

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

11.1 Purification of genomic DNA:

11.1.1 Isolate genomic DNA with your desired method of choice.

Several different methods are available for isolating genomic DNA. We recommend using Genomic DNA Isolation Kit (ab65358) for simplicity. Alternatively, performing guanidine/detergent lysis is simple and it gives highly purified genomic DNA for the ARP-based abasic sites detection.

Δ Note: avoid heating the DNA solution during the purification process as it will introduce AP sites.

11.1.2 Determine the concentration and purity of the purified genomic DNA.

Δ Note: 50 µg/mL genomic DNA = 1 OD260 nm. Ratio of OD_{260}/OD_{280} of highly purified DNA solution is ≥ 1.7. Protein contamination in the sample solution may cause a positive error.

11.1.3 Dilute purified genomic DNA to 100 µg/mL in TE buffer.

11.2 ARP reaction:

11.2.1 Mix 5 µL of purified genomic DNA (100 µg/mL) with 5 µL of ARP solution (Step 9.2) in a microcentrifuge tube and incubate 1 hour at 37°C.

11.2.2 Add 90 µL TE buffer and 1 µL Glycogen Solution (Step 9.4) to each sample tube. Mix well by pipetting up and down.

11.2.3 Add 10 µL Sodium Acetate Solution (Step 9.8) to each sample tube. Mix well by pipetting up and down.

11.2.4 Add 300 µL absolute ethanol (EtOH 100%) to each tube and mix well by vortexing briefly.

11.2.5 Incubate mix at -20°C for 30 minutes.

11.2.6 Centrifuge tubes for 10-20 minutes at 14,000 x*g* in a cold microcentrifuge. Discard supernatant carefully.

11.2.7 Carefully wash DNA pellet 3 times in 70% ethanol. Quickly spin to remove the trace amount of ethanol.

11.2.8 Air dry the DNA pellet for 5 minutes.

11.2.9 Dissolve the DNA pellet in 10-50 µL TE buffer and determine the DNA concentration with your method of choice (assume 70% recovery).

Δ Note: ARP-derived DNA can be stored at -20°C for up to one year.

11.2.10 Dilute ARP-derived DNA sample to 1 µg/mL in TE buffer.

11.3 Reaction wells set up:

In the provided DNA high-binding plate (Step 9.5), add the following standard and sample amounts:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 50 µL ARP-derived DNA samples (1 µg/mL).

11.4 Determination of AP sites in DNA:

11.4.1 Add 50 µL DNA Binding Solution (Step 9.4) to each well. Mix well by pipetting up and down and incubate at room temperature for 2 hours or overnight on an orbital shaker.

11.4.2 Wash microwell strips 3 times in 250 µL 1X Wash Buffer with thorough aspiration between each wash.

11.4.3 After last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess wash buffer.

11.4.4 Add 100 µL diluted Streptavidin-Enzyme Conjugate (Step 9.10) to each well. Incubate plate at 37°C for 1 hour.

11.4.5 Wash microwell strips 3 times in 250 µL 1X Wash Buffer with thorough aspiration between each wash.

11.4.6 After last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess wash buffer.

11.4.7 Add 100 µL Substrate Solution (Step 9.11) to each well, including blank wells. Incubate at room temperature 5-20 minutes on an orbital shaker.

Δ Note: Add the stop solution when you see a nice blue gradient with the standard curve. Longer incubation times (also shorter than 20 minutes) might lead to precipitation after addition of the stop solution. The presence of precipitate will interfere with the measurement and might lead to inaccurate absorbance values.

11.4.8 Stop the reaction by adding 100 µL of Stop Solution (Step 9.9) into each well, including blank wells.

11.4.9 Measure output immediately on a microplate reader at OD 450 nm (primary wavelength).

12. Calculations

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

12.1 Average the duplicate reading for each standard and sample.

12.2 Subtract the mean absorbance value of the blank (Standard #8) from all standard and sample readings. This is the corrected absorbance.

12.3 Plot the corrected absorbance values for each standard as a function of AP sites per 10^5 bp.

12.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

12.5 Apply the corrected sample OD reading to the standard curve to get the number of AP sites/10^5 bp in your sample wells.

12.6 Compare the number of AP sites in treated samples vs control untreated samples to determine the level of DNA damage.

13. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

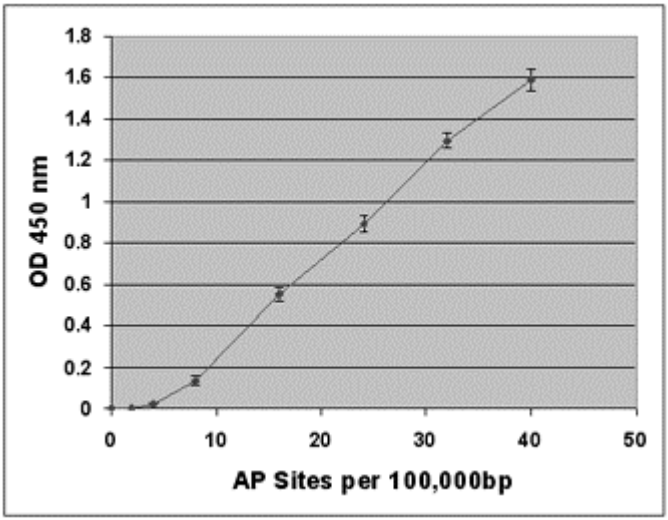


Figure 1. Typical ARP-DNA standard calibration curve.

14. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

15. FAQs

Q. Can I use this product to study bacterial damage?

A. Yes. The assay uses purified DNA, so it suitable for use with DNA extracted from bacteria.

Q. Can I use less than 100 µg/mL of DNA?

A. Yes, it is possible to use less than 100 µg/mL DNA, as long as the resulting APR-derived DNA is at a minimum concentration of 1 µg/mL with at least 50 µL following the ARP reaction.

Q. Can I use TE buffer pH 8.0 to prepare the genomic DNA?

A. Yes, it is fine to use DNA samples in TE buffer at pH 8.0.

16. Notes

Technical Support

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