EpiQuik™ 8-OhG RNA Damage Quantification Direct Kit (Colorimetric)

Base Catalog # P-6008

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ 8-OhG RNA Damage Quantification Direct Kit (Colorimetric) is suitable for directly detecting oxidative RNA damage (8-OhG) status using RNA isolated from any species such as mammals, plants, fungi, bacteria, and viruses in a variety of forms including, but not limited to, cultured cells, fresh and frozen tissues, paraffin-embedded tissues, and body fluid samples.

Input RNA: The amount of RNA for each assay can be 100 ng to 300 ng. For optimal quantification, the input RNA amount should be 300 ng, as basal 8-OhG is generally less than 0.01% of total RNA.

Starting Material: Starting materials can include various tissue or cell samples such as cells from flask or microplate cultured cells, fresh and frozen tissues, paraffin-embedded tissues, blood, body fluid samples, etc.

Internal Control: Both negative and positive RNA controls are provided in this kit. A standard curve can be performed (range: 5 to 200 pg of 8-OhG) or a single quantity of 8-OhG can be used as a positive control. Because 8-OhG content can vary from tissue to tissue, and from normal and diseased states, or vary under treated and untreated conditions, it is advised to run replicate samples to ensure that the signal generated is validated. This kit will allow the user to quantify an absolute amount of 8-OhG and determine the relative 8-OhG states of two different RNA samples.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	48 Assays Cat. #P-6008-48	96 Assays Cat. #P-6008-96	Storage Upon Receipt
WB (10X Wash Buffer)	14 ml	28 ml	4°C
BS (Binding Solution)	5 ml	10 ml	RT
NC (Negative Control, 10 µg/ml)*	10 µl	20 µl	–20°C
PC (Positive Control, 8-OhG 0.2 µg/ml)*	10 µl	20 µl	–20°C
OCA (OhG Capture Antibody, 100 X) *	28 µl	55 µl	4°C
ODA (OhG Detection Antibody, 1000 X)*	6 µl	12 µl	–20°C
ES (Enhancer Solution)*	5 µl	10 µl	–20°C
DS (Developer Solution)	5 ml	10 ml	4°C
SS (Stop Solution)	5 ml	10 ml	RT
8-Well Assay Strips (With Frame)	6	12	4°C
User Guide	1	1	RT

** Spin the solution down to the bottom prior to use.*

***Note**: The **NC** (Negative Control) is an oligos containing no 8-OhG. The **PC** (Positive Control) is an 8-OhG oligos and is normalized to have 100% of 8-OhG.*

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **NC**, **PC**, **OCA, ODA**, and **ES** at –20°C away from light; (2) Store **WB**, **DS**, and **8-Well Assay Strips** at 4°C away from light; (3) Store remaining components (**BS** and **SS**) at room temperature away from light.

***Note**: Check if wash buffer, **WB**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.*

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Adjustable pipette
- ☐ Aerosol resistant pipette tips
- ☐ Microplate reader capable of reading absorbance at 450 nm
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Incubator for 37°C incubation
- ☐ Plate seal or Parafilm M
- ☐ Distilled water
- ☐ 1X TE buffer pH 7.5 to 8.0
- ☐ Isolated RNA of interest

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ 8-OhG RNA Damage Quantification Direct Kit (Colorimetric) is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The EpiQuik™ 8-OhG RNA Damage Quantification Direct Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiQuik™ 8-OhG RNA Damage Quantification Direct Kit (Colorimetric) and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

8-hydroxyguanosine (8-OhG or 8-oxoG) is an oxidized derivative of guanosine and is generated by hydroxyl radicals, singlet oxygen, and one-electron oxidants in cellular RNA. As a modified nucleoside base, 8-OhG is considered important not only because of its abundance but also because of its mutagenic mispairing that occurs during RNA synthesis and may induce errors in gene expression. 8-OhG also participates in epigenetic regulation of gene activation/repression by affecting chromatin modification. Unlike oxidative DNA that is quickly repaired by base excision repair pathways intracellularly, oxidized RNA can remain in the cell for hours after a short insult of oxidative stress, and these oxidative marks on RNA could be identified as an early indicator of cell death.

Evidence shows that increased levels of 8-OhG are closely correlated with exposure to harmful environmental factors such as ionizing radiation, industrial chemicals, air pollution, cigarette smoking, and cancer chemotherapy. It has also been demonstrated that increased concentrations of 8-OhG are pathogenically linked to a variety of age-associated diseases, including cancer, coronary heart disease, diabetes, and neurodegenerative diseases such as Alzheimer's disease. Compared with the urine 8-OhG assay that mainly reflects the balance between oxidative damage and the repair rate of the whole body, directly quantifying the 8-OhG content in different cells/tissues in normal and disease states would allow tissue-specific oxidative damage of RNA to be identified. Therefore, more useful information for better understanding oxidative damage-disease relationships, which benefits diagnostics and therapeutics of the disease, can be obtained.

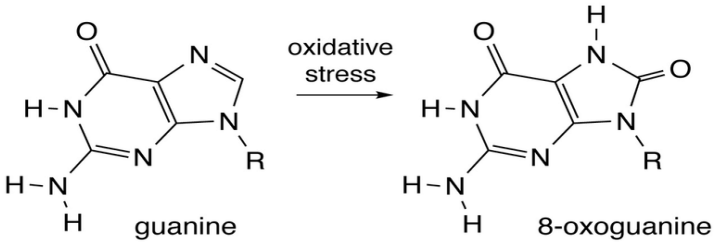

Fig 1: The oxidation of guanine to form 8-oxoguanine (Lanier KA et al: J Mol Evol, 2017)

Several chromatography-based techniques such as HPLC-ECD and LC-MS are used for detecting 8-OhG in tissues and cells. However, these methods are time consuming and have low throughput with high costs. The currently used competitive ELISA methods are also not conveniently applicable for cell/tissue 8-OhG detection because they are less accurate and cannot use intact RNA isolated from cells or tissues directly. To address these problems, EpigenTek offers the EpiQuik™ 8-OhG RNA Damage Quantification Direct Kit (Colorimetric) which uses a unique procedure that directly quantifies 8-OhG in cells/tissues. The kit has the following advantages and features:

- Colorimetric assay with easy-to-follow steps for convenience and speed. The entire procedure can be completed within 3 hours.
- High sensitivity, of which the detection limit can be as low as 2 pg of 8-OhG.
- High specificity by detecting only 8-OhG within the indicated concentration range of the sample RNA.
- Direct detection of 8-OhG using intact RNA, which eliminates interference from high molecular weight compounds, such as carbohydrates and proteins that are often seen in competitive 8-OhG assays.
- Highly convenient assay with direct use of RNA isolated from cells or tissues, no need for RNA digestion or hydrolysis.
- Universal positive and negative controls are included, which are suitable for quantifying 8-OhG from any species.
- Strip-well microplate format makes the assay flexible for manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The EpiQuik™ 8-OhG RNA Damage Quantification Direct Kit (Colorimetric) contains all reagents necessary for the quantification of Oxidative RNA damage (8-OhG). In this assay, RNA is bound to strip wells that are specifically treated to have a high RNA affinity. 8-OhG is detected using capture and detection antibodies. The detected signal is enhanced and then quantified colorimetrically by reading the absorbance in a microplate spectrophotometer. The amount of 8-OhG is proportional to the OD intensity measured.

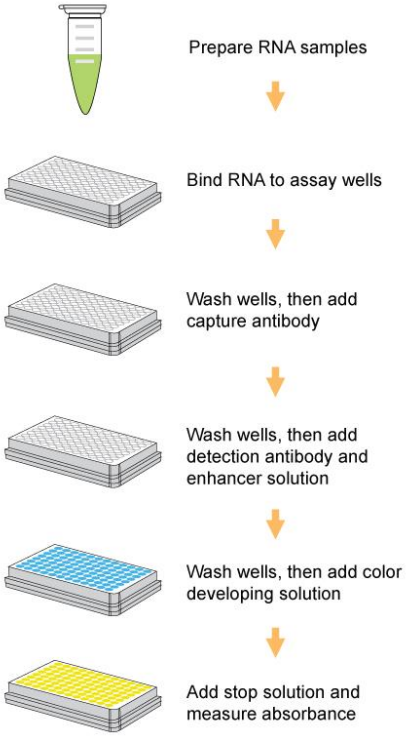

Fig 2: Schematic procedure of the EpiQuik™ 8-OhG RNA Damage Quantification Direct Kit (Colorimetric)

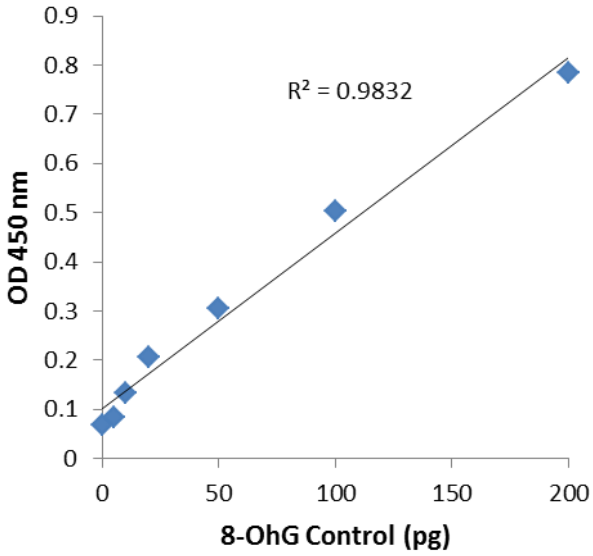

Fig 3: 8-OhG standard control was added into the assay wells at different concentrations and then measured with the EpiQuik™ 8-OhG RNA Damage Quantification Direct Kit (Colorimetric)

ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

1. Starting Materials

Input RNA Amount: RNA amount can range from 100 ng to 300 ng per reaction. An optimal amount is 300 ng per reaction. RNA should be high quality and relatively free of DNA. DNase I can be used to remove DNA. RNA should be eluted in RNase-free water.

RNA Isolation: You can use your method of choice for RNA isolation. EpigenTek offers a series of RNA isolation kits for your convenience (see “Related Products” section).

RNA Storage: Isolated RNA should be stored at -20°C (short term) or -80°C (long term) until use.

2. Buffer and Solution Preparation

a. Preparation of **Diluted WB** (1X Wash Buffer):

48-Assay Kit: Add 13 ml of **WB** (10X Wash Buffer) to 117 ml of distilled water (final pH 7.2-7.5).

96-Assay Kit: Add 26 ml of **WB (**10X Wash Buffer) to 234 ml of distilled water (final pH 7.2-7.5).

This **Diluted WB** (1X Wash Buffer) can now be stored at 4°C for up to six months.

b. Prepare **Diluted OCA** (OhG Capture Antibody Solution):

Dilute **OCA** (OhG Capture Antibody) with **Diluted WB** at a ratio of 1:100 [i.e., add 1 µl of **OCA** to 99 µl of **Diluted WB**]. About 50 µl of this **Diluted OCA** will be required for each assay well.

The anticipated approximate volumes of reagents needed are reflected below for this assay.

Reagents	1 well	8 wells (1 strip)	16 wells (2 strips)	48 wells (6 strips)	96 wells (12 strips)
Diluted WB	2.5 ml	20 ml	40 ml	120 ml	240 ml
BS	100 µl	800 µl	1600 µl	4800 µl	9600 µl
OCA	50 µl	400 µl	800 µl	2400 µl	4800 µl
Detection Complex	50 µl	400 µl	800 µl	2400 µl	4800 µl
DS	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
SS	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
NC	N/A	0.5 µl – 1 µl	0.5 µl – 2 µl	1 µl – 4 µl	2 µl – 8 µl
PC	N/A	0.5 µl – 1 µl	0.5 µl – 2 µl	1 µl – 4 µl	2 µl – 8 µl

3. Preparation of Diluted Positive Control

Single Point Control Prep: Dilute **PC** (Positive Control) with 1X TE to 100 pg/µl (1 µl **PC** + 1 µl TE).

Suggested Standard Curve Prep: Prepare 6 different concentrations with the stock **PC** and 1X TE into 5, 10, 20, 50, 100 and 200 pg/µl according to the following dilution chart:

Tube	PC (200 pg/µl)	1X TE	Resulting PC Concentration
1	1.0 µl	39.0 µl	5 pg/µl
2	1.0 µl	19.0 µl	10 pg/µl
3	1.0 µl	9.0 µl	20 pg/µl
4	1.0 µl	3.0 µl	50 pg/µl
5	2.0 µl	2.0 µl	100 pg/µl
6	4.0 µl	0.0 µl	200 pg/µl

Note: *Keep each of the diluted solutions (except **Diluted WB**) on ice until use. Any remaining diluted solutions, other than **Diluted WB**, should be discarded if not used within the same day.*

4. RNA Binding

a. Predetermine the number of strip wells required for your experiment. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

b. For negative control wells: Add 100 µl of **BS** (Binding Solution) and 1 µl of **NC** (Negative Control).

c. For positive control wells: Add 100 µl of **BS** (Binding Solution) and 1 µl of **PC** (Positive Control) at different concentrations (5-200 pg/ µl) to generate a standard curve (see note below).

d. For sample wells: Add 100 µl of **BS** (Binding Solution) and 300 ng of your sample RNA (3 µl).

Note: *(1) To reduce cross variation between replicates, it is important to load the wells in vertical formation according to the plate layout depicted on page 9. The positive controls should be assayed in parallel with the samples in the same plate and a new positive control standard curve should be generated for each assay. (2) For optimal binding and to reduce pipetting error, sample RNA volume added should be 2 µl or more, but should not exceed 6 µl. (3) To ensure that* ***NC****,* ***PC****, and sample RNA are completely added into the wells, the RNA should be mixed well before use and the pipette tip should be placed into the* ***BS*** *solution in the well and aspirated in/out 1-2 times. Changing the tips each time when adding the sample will increase sample volume accuracy added into each well.*

e. Mix solution by gently tilting from side to side or by gently shaking the plate several times to ensure the solution coats the bottom of the well evenly. Cover strip plate with plate seal or Parafilm M and incubate at 37°C for 75 minutes.

f. Remove the **BS** Binding Solution from each well. Wash each well with 150 µl of the **Diluted WB** (1X Wash Buffer) each time for two times.

5. 8-OhG RNA Capture

a. Add 50 µl of the **Diluted OCA (**OhG Capture Antibody) to each well, then cover and incubate at room temperature for 60 min.

b. During the last 10 minutes of sample incubation, prepare the **Detection Complex**: In each 1 ml of **Diluted WB** (1X Wash Buffer) add 1 µl of **ODA** OhG (Detection Antibody) and 0.5 µl of **ES** (Enhancer Solution). Mix well.

c. Remove the **Diluted OCA** solution from each well after 60 min incubation.

d. Wash each well with 150 µl of the **Diluted WB** (1X Wash Buffer) each time for three times.

e. Add 50 µl of the **Detection Complex** to each well, then cover and incubate at room temperature for 30 min.

f. Remove the **Detection Complex** solution from each well.

g. Wash each well with 150 µl of the **Diluted WB** (1X Wash Buffer) each time for five times.

6. Signal Detection

a. Add 100 µl of **DS** (Developer Solution) to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The **DS** solution will turn blue in the presence of sufficient 8-OhG.

b. Add 100 µl of **SS (Stop Solution)** to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding **SS** and the absorbance should be read on a microplate reader at 450 nm within 2 to 15 min.

Note: *If the strip-well plate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.*

7. 8-OhG Calculation

Relative Quantification: To determine the relative 8-OhG status of two different RNA samples, a simple calculation for the percentage of 8-OhG in total RNA can be carried out using the following formula:

$$\textit{8-OhG \%} = \frac{\textit{(Sample OD – NC OD) ÷ S}}{\textit{(PC OD – NC OD) ÷ P}} \times \textit{100\%}$$

S is the amount of input sample RNA in ng.
P is the amount of input positive control (**PC**) in ng.

Example calculation:

Average OD450 of NC is 0.080
Average OD450 of PC is 0.580
Average OD450 of Sample is 0.180
S is 300 ng
P is 0.1 ng (100 pg)

$$\text{8-OhG \%} = \frac{(0.180 - 0.08) \div 300}{(0.580 - 0.080) \div 0.1} \times 100\% = \mathbf{0.0067\%}$$

Absolute Quantification: To quantify the absolute amount of 8-OhG using an accurate calculation, first generate a standard curve and plot the OD values versus the amount of **PC** at each concentration point. Next, determine the slope (OD/ng) of the standard curve using linear regression (*Microsoft Excel's* linear regression functions are suitable for such calculation) and also determine the most linear part (at least 4 concentration points including 0 point) of the standard curve for optimal slope calculation. Now calculate the amount and percentage of 8-OhG in your total RNA using the following formulas:

$$\textit{8-OhG (ng)} = \frac{\textit{Sample OD – NC OD}}{\textit{Slope}}$$

$$\textit{8-OhG \%} = \frac{\textit{8-OhG Amount (ng)}}{\textit{S}} \times \textit{100\%}$$

S is the amount of input sample RNA in ng.

Example calculation:

Average OD450 of NC is 0.08
Average OD450 of sample is 0.180
Slope is 4 OD/ng
S is 300 ng

$$\text{8-OhG (ng)} = \frac{0.180 - 0.080}{4} = \mathbf{0.025\ ng}$$

$$\text{8-OhG \%} = \frac{0.025}{300} \times 100\% = \mathbf{0.0083\%}$$

SUGGESTED STRIP WELL SETUP

Table 1. The suggested strip-well plate setup using a single point positive control in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	NC	Sample	Sample	Sample	Sample	Sample
B	NC	Sample	Sample	Sample	Sample	Sample
C	PC 100 pg/µl	Sample	Sample	Sample	Sample	Sample
D	PC 100 pg/µl	Sample	Sample	Sample	Sample	Sample
E	Sample	Sample	Sample	Sample	Sample	Sample
F	Sample	Sample	Sample	Sample	Sample	Sample
G	Sample	Sample	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

Table 2. The suggested strip-well plate setup for standard curve preparation in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	NC	PC 50 pg/µl	Sample	Sample	Sample	Sample
B	NC	PC 50 pg/µl	Sample	Sample	Sample	Sample
C	PC 5 pg/µl	PC100 pg/µl	Sample	Sample	Sample	Sample
D	PC 5 pg/µl	PC 100 pg/µl	Sample	Sample	Sample	Sample
E	PC 10 pg/µl	PC 200 pg/µl	Sample	Sample	Sample	Sample
F	PC 10 pg/µl	PC 200 pg/µl	Sample	Sample	Sample	Sample
G	PC 20 pg/µl	Sample	Sample	Sample	Sample	Sample
H	PC 20 pg/µl	Sample	Sample	Sample	Sample	Sample

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal in both the positive control and sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order and if any steps in the protocol may have been omitted by mistake.
	The well is incorrectly washed before RNA binding.	Ensure the well is not washed prior to adding the positive control and sample.
	The bottom of the well is not completely covered by the **BS** (Binding Solution).	Ensure the solution coats the bottom of the well by gently tilting from side to side or shaking the plate several times.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol is followed correctly.
	Insufficient input materials.	Ensure that a sufficient amount of positive control (> 100 pg) and sample (300 ng) is added into the wells.
	Incorrect absorbance reading.	Check if the appropriate absorbance wavelength (450 nm) is used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly secured after each opening or use.

No signal or weak signal in only the positive control wells	The positive control is insufficiently added to the well in Step 4c.	Ensure a sufficient amount of positive control RNA is added.
	The **PC** (Positive Control) is degraded due to improper storage conditions.	Follow the Shipping & Storage guidelines of this User Guide for storage of **PC**.
High background present in the negative control wells	Insufficient washing of wells.	Check if washing recommendations at each step are performed according to the protocol.
	Contaminated by sample or positive control.	Ensure the well is not contaminated by the sample or positive control RNA or from the use of contaminated tips.
	Incubation time is too long.	The incubation time at Step 5a should not exceed 2 h.
	Over development of color.	Decrease the development time in Step 6a before adding **SS** (Stop Solution) in Step 6b.
No signal or weak signal only in sample wells	RNA sample is not properly extracted or purified.	Ensure the RNA sample is in good quality. 260/280 ratio should be >1.9 with no or minimal protein and DNA contamination.
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of RNA is used as indicated in Step 4.
	Little or no 8-OhG in the sample.	N/A
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the washing guidelines. Make sure the residue of the washing buffer is removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development solution or stop solution is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well H or from well 1 to well 12).

RELATED PRODUCTS

RNA Sample Preparation

P-9105 EpiQuik™ Total RNA Isolation Fast Kit
P-9106 EpiQuik™ Magbeads Quick RNA Isolation Kit

Methylated RNA Quantification

P-9005 EpiQuik™ m6A RNA Methylation Quantification Kit (Colorimetric)
P-9008 EpiQuik™ m6A RNA Methylation Quantification Kit (Fluorometric)
P-9009 MethylFlash™ 5-mC RNA Methylation ELISA Easy Kit (Fluorometric)
P-9015 MethylFlash™ Urine N^6-methyladenosine (m6A) Quantification Kit (Colorimetric)

DNA Damage and Repair

OP-0001 EpiQuik™ Superoxide Dismutase Activity/Inhibition Assay Kit (Colorimetric)
P-6001 EpiQuik™ In Situ DNA Damage Assay Kit
P-6003 EpiQuik™ 8-OHdG DNA Damage Quantification Direct Kit (Colorimetric)
P-6004 EpiQuik™ 8-OHdG DNA Damage Quantification Direct Kit (Fluorometric)
P-6005 EpiQuik™ In-situ and Ex-situ Hydrogen Peroxide (H_2O_2) Assay Kit

DNA Damage and Repair Antibodies: (See https://www.epigentek.com/catalog/dna-damage-repair-antibodies-c-35_70.html)