EpiQuik™ Nitrosative (8-Nitroguanine) DNA/RNA Damage Quantification Kit (Colorimetric)

Base Catalog # P-6009

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ Nitrosative (8-Nitroguanine) DNA/RNA Damage Quantification Kit (Colorimetric) is suitable for detecting nitrosative DNA/RNA damage (8-nitroguanine, 8-NG) status directly using DNA /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 DNA/RNA: The amount of total DNA or total RNA for each assay can be 100 ng to 300 ng. For optimal quantification, the input DNA and RNA amount should be 300 ng, as basal 8-NG is generally less than 0.01% of total DNA or 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 controls are provided in this kit. A standard curve can be performed (range: 5 to 200 pg of 8-NG) or a single quantity of 8-NG can be used as a positive control. Because 8-NG 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-NG and determine the relative 8-NG states of two different DNA/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-6009-48	96 Assays Cat. #P-6009-96	Storage Upon Receipt
WB (10X Wash Buffer)	14 ml	28 ml	4°C
BS (Binding Solution)	5 ml	10 ml	RT
8-NC (Negative Control, 10 µg/ml)*	10 µl	20 µl	–20°C
8-PC (Positive Control, 8-NG 0.1 µg/ml)*	12 µl	24 µl	–20°C
CA (Capture Antibody, 1000 X) *	4 µl	8 µl	4°C
DA (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

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

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

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 8-**NC**, **8-PC**, **DA**, and **ES** at –20°C away from light; (2) Store **WB**, **CA**, **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 DNA/RNA of interest

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ Nitrosative (8-Nitroguanine) DNA/RNA Damage Quantification 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.

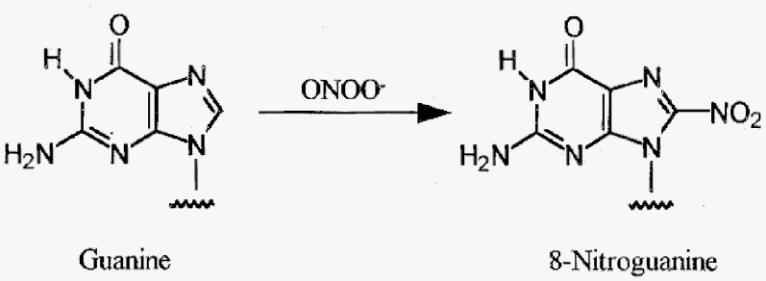
Usage Limitation: The EpiQuik™ Nitrosative (8-Nitroguanine) DNA/RNA Damage Quantification Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

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

A BRIEF OVERVIEW

8-nitroguanine (8-NG) is a mutagenic nitrative DNA/RNA lesion caused by reactive nitrogen species (RNS). As a modified nucleoside base, 8-NG is considered important because of its abundance and its mutagenic potential through G-to-T transversion mutations upon replication of DNA. 8-NG also participates in epigenetic regulation of gene activation/repression by causing breaks in DNA to form single-stranded DNA.

It has also been demonstrated that increased concentrations of 8-NG are pathogenically linked to various inflammation-associated diseases, including cancer. Comparative urine/serum 8-NG assays mainly reflect the balance between nitrosative damage and the repair rate of the whole body. However, quantifying 8-NG content directly in different cells/tissues of normal/disease states allows tissue-specific nitrosative damage of DNA/RNA to be identified. Thus, this method provides more useful information about nitrosative damage-disease relationships to benefit disease diagnostics and therapeutics.



Published in *Frontiers in Bioscience*: a journal and virtual library 1997. **Interactions between superoxide and nitric oxide: implications in DNA damage and mutagenesis.** D. Jourd'heuil, D. Kang, M. Grisham.

Several chromatography-based techniques such as HPLC-ECD and LC-MS are used for detecting 8-NG 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-NG detection because they are less accurate and cannot directly use intact DNA or RNA isolated from cells or tissues. To address these problems, EpigenTek offers the EpiQuik™ Nitrosative (8-Nitroguanine) DNA/RNA Damage Quantification Kit (Colorimetric), which uses a unique procedure to directly quantify 8-NG 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 with a detection limit as low as 2 pg of 8-NG.
- High specificity by detecting only 8-NG without cross-reactivity to 8-OHdG, 8-OHG, dG, guanine, within the indicated concentration range of the sample DNA or RNA.
- Direct detection of 8-NG using intact DNA or RNA, which eliminates interference from high molecular weight compounds, such as carbohydrates and proteins that are often seen in competitive 8-NG assays.
- Detection level is highly correlated with and close to HPLC or LC-MS analysis.
- Highly convenient assay with direct use of DNA or RNA isolated from cells or tissues, no need for DNA/ RNA digestion or hydrolysis.
- Universal positive and negative controls are included, which are suitable for quantifying 8-NG 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™ Nitrosative (8-Nitroguanine) DNA/RNA Damage Quantification Kit (Colorimetric) contains all reagents necessary for the quantification of nitrosative (8-nitroguanine) DNA/RNA. In this assay, DNA/RNA is bound to strip wells that are specifically treated to have a high nucleic acid binding affinity. 8-NG 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-NG is proportional to the OD intensity measured.

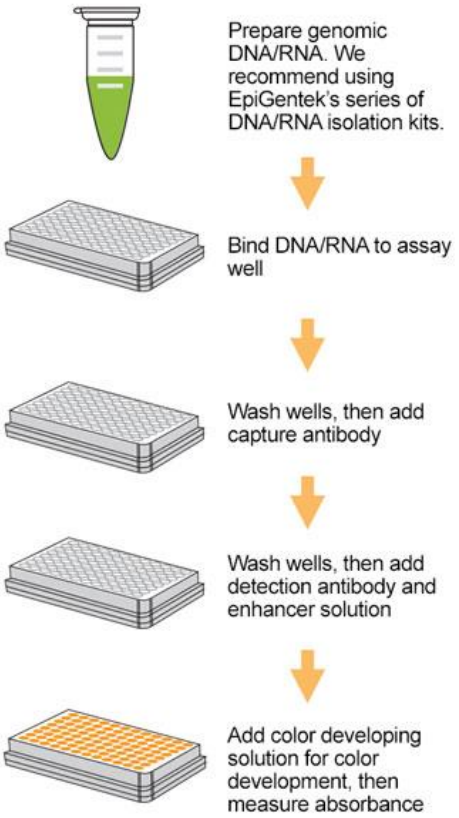


Fig 2. Schematic procedure of the EpiQuik™ Nitrosative (8-Nitroguanine) DNA/RNA Damage Quantification Kit (Colorimetric)

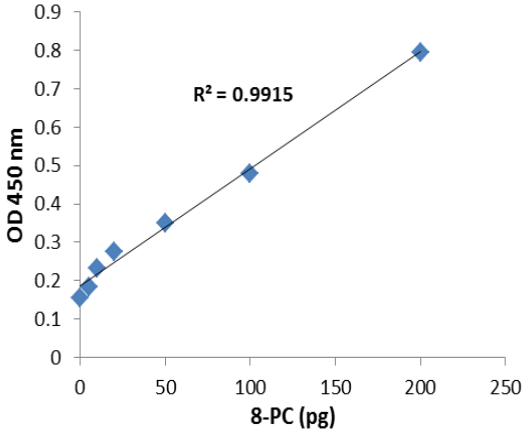


Fig 3. 8-NG standard control was added into the assay wells at different concentrations and then measured with the EpiQuik™ Nitrosative (8-Nitroguanine) DNA/RNA Damage Quantification Kit (Colorimetric)

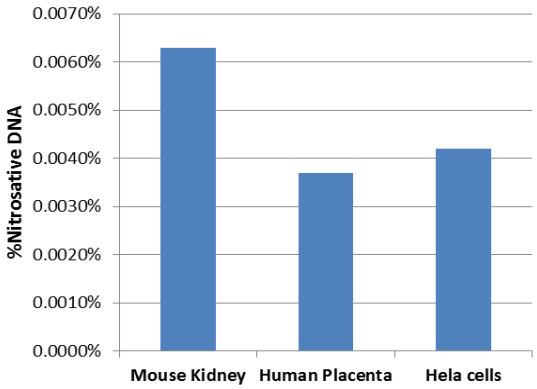


Fig 4. Percentage of 8-NG in different tissue/cells measured with the EpiQuik™ Nitrosative (8-Nitroguanine) DNA/RNA Damage Quantification Kit (Colorimetric)

ASSAY PROTOCOL

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

1. Starting Materials

Input DNA/RNA Amount: DNA/RNA amount can range from 100 ng to 300 ng per reaction. An optimal amount is 300 ng per reaction. Starting DNA/RNA may be in water or in a buffer such as TE.

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

DNA/RNA Storage: Isolated DNA/RNA can be stored at –20°C until use.

2. Buffer and Solution Preparation

a. Preparation of **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 CA** (Capture Antibody) Solution:

Dilute **CA** (Capture Antibody) with **Diluted WB** (1X Wash Buffer) at a ratio of 1:1000 (i.e., add 1 µl of **CA** to 1000 µl of **Diluted WB**). About 50 µl of this **Diluted CA** will be required for each assay well.

c. Prepare **Diluted DA** (Detection Antibody Solution):

Dilute **DA** (Detection Antibody) with **Diluted WB** (1X Wash Buffer) at a ratio of 1:1000 (i.e., add 1 µl of **DA** to 1000 µl of **Diluted WB**). About 50 µl of this **Diluted DA** will be required for each assay well.

d. Prepare **Diluted ES** (Enhancer Solution):

Dilute **ES** (Enhancer Solution) with **Diluted WB** (1X Wash Buffer) at a ratio of 1:5000 (i.e., add 1 µl of **ES** to 5000 µl of **Diluted WB**). About 50 µl of this **Diluted ES** will be required for each assay well.

e. Preparation of **Diluted Positive Control**:

Single Point Control Prep: Dilute 8-**PC** (Positive Control) with 1X TE to 50 pg/µl (1 µl 8-**PC** + 1 µl TE).

Suggested Standard Curve Prep: Prepare 6 different concentrations with the stock 8-**PC** and 1X TE into 2.5, 5, 10, 20, 50, and 100 pg/µl according to the following dilution chart:

Tube	8-PC (100 pg/µl)	1X TE	Resulting 8-PC Concentration
1	1.0 µl	39.0 µl	2.5 pg/µl
2	1.0 µl	19.0 µl	5 pg/µl
3	1.0 µl	9.0 µl	10 pg/µl
4	1.0 µl	3.0 µl	20 pg/µl
5	2.0 µl	2.0 µl	50 pg/µl
6	4.0 µl	0.0 µl	100 pg/µl

Note: *Keep each diluted solution, except* ***Diluted WB*** *(1X Wash Buffer), on ice until use. Other than* ***Diluted WB****, discard any remaining diluted solutions if not used within the same day.*

3. DNA/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. Add 80 µl of **BS** (Binding Solution) to each well.

c. Add 2 µl of 8-**NC** (Negative Control), 2 µl of **Diluted** 8-**PC** (see note below), and 300 ng of your sample DNA/RNA (1-8 µl) into the designated wells depicted in Table 1 or Table 2. Mix solution by gently tilting from side to side or shaking the plate several times. Ensure the solution coats the bottom of the well evenly.

Note: *(1) For a single point control, add 2 µl of* ***8-PC*** *(Positive Control) at a concentration of 50 pg/µl as prepared in Step 2e; For the standard curve, add 2 µl of* ***Diluted 8-PC*** *at concentrations of 2.5 to 100 pg/µl (see the chart in Step 2). The final amounts should be 5, 10, 20, 50, 100 and 200 pg per well. (2) For optimal binding, sample DNA/RNA volume added should not exceed 8 µl.*

d. Cover strip plate with plate seal or Parafilm M and incubate at 37°C for 75-90 min.

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

4. 8-NG Capture

a. Add 50 µl of the **Diluted CA** to each well, then cover and incubate at room temperature for 60 min.

b. Remove the **Diluted CA** solution from each well.

c. Wash each well with 150 µl of the **Diluted WB** each time for three times.

d. Add 50 µl of the **Diluted DA** to each well, then cover and incubate at room temperature for 30 min.

e. Remove the **Diluted DA** solution from each well.

f. Wash each well with 150 µl of the **Diluted WB** each time for four times.

g. Add 50 µl of the **Diluted ES** to each well, then cover and incubate at room temperature for 30 min.

h. Remove the **Diluted ES** solution from each well.

i. Wash each well with 150 µl of the **Diluted WB** each time for five times.

5. 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-NG.

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.*

6. 8-NG Calculation

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

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

S is the amount of input sample DNA/RNA in ng.
P is the amount of input positive control (**8-PC**) in ng.

Example calculation:

Average OD450 of 8-NC is 0.085
Average OD450 of 8-PC is 0.485
Average OD450 of Sample is 0.145
S is 300 ng
P is 0.1 ng (100 pg)

$$\text{8-NG \%} = \frac{(0.145 - 0.085) \div 300}{(0.485 - 0.085) \div 0.1} \times 100\% = \mathbf{0.005\%}$$

Absolute Quantification: To quantify the absolute amount of 8-NG using an accurate calculation, first generate a standard curve and plot the OD values versus the amount of 8-**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 (include at least 4 concentration points) of the standard curve for optimal slope calculation. Now calculate the amount and percentage of 8-NG in your total DNA/RNA using the following formulas:

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

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

S is the amount of input sample DNA/RNA in ng.

Example calculation:

Average OD450 of 8-NC is 0.085
Average OD450 of sample is 0.145
Slope is 4 OD/ng
S is 300 ng

$$\text{8-NG (ng)} = \frac{0.145 - 0.085}{4} = \mathbf{0.015\ ng}$$

$$\text{8-NG \%} = \frac{0.015}{300} \times 100\% = \mathbf{0.005\%}$$

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	8-NC	8-NC	Sample	Sample	Sample	Sample
B	8-PC	8-PC	Sample	Sample	Sample	Sample
C	Sample	Sample	Sample	Sample	Sample	Sample
D	Sample	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	8-NC	8-NC	Sample	Sample	Sample	Sample
B	8-PC 2.5 pg/µl	8-PC 2.5 pg/µl	Sample	Sample	Sample	Sample
C	8-PC 5 pg/µl	8-PC 5 pg/µl	Sample	Sample	Sample	Sample
D	8-PC 10 pg/µl	8-PC 10 pg/µl	Sample	Sample	Sample	Sample
E	8-PC 20 pg/µl	8-PC 20 pg/µl	Sample	Sample	Sample	Sample
F	8-PC 50 pg/µl	8-PC 50 pg/µl	Sample	Sample	Sample	Sample
G	8-PC 100 pg/µl	8-PC 100 pg/µl	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

SUGGESTED WORKING BUFFER AND SOLUTION SETUP

Table 3. Approximate amount of required buffers and solutions for defined assay wells based on the protocol.

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	80 µl	640 µl	1300 µl	3900 µl	8000 µl
Diluted CA	50 µl	400 µl	800 µl	2400 µl	4800 µl
Diluted DA	50 µl	400 µl	800 µl	2400 µl	4800 µl
Diluted ES	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
8-NC	N/A	0.5 µl – 1 µl	0.5 µl – 2 µl	1 µl – 4 µl	2 µl – 8 µl
8-PC	N/A	0.5 µl – 1 µl	0.5 µl – 2 µl	1 µl – 4 µl	2 µl – 8 µl

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 DNA/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 (> 50 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 3c.	Ensure a sufficient amount of positive control is added.
	The **8-PC** (Positive Control) is degraded due to improper storage conditions.	Follow the Shipping & Storage guidelines of this User Guide for storage of 8-**PC** (Positive Control).
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 DNA/RNA or from the use of contaminated tips.
	Incubation time is too long.	The incubation time at Step 3d should not exceed 2 h.
	Over development of color.	Decrease the development time in Step 5a before adding **SS** (Stop Solution) in Step 5b.
No signal or weak signal only in sample wells	Sample is not properly extracted or purified.	Ensure the sample is in good quality. 260/280 ratio should be >1.7 for DNA and >1.9 for RNA.
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of DNA/RNA is used as indicated in Step 3.
	Little or no 8-NG contained 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 G or from well 1 to well 12).

RELATED PRODUCTS

DNA/RNA Sample Preparation

P-1003 FitAmp™ General Tissue Section DNA Isolation Kit
P-1004 FitAmp™ Plasma/Serum DNA Isolation Kit
P-1006 DNA Concentrator Kit
P-1009 FitAmp™ Paraffin Tissue Section DNA Isolation Kit
P-1017 FitAmp™ Urine DNA Isolation Kit
P-1018 FitAmp™ Blood and Cultured Cell DNA Extraction Kit
P-9105 EpiQuik™ Total RNA Isolation Fast Kit
P-9106 EpiQuik™ Magbeads Quick RNA Isolation Kit

Methylated and Hydroxymethylated DNA Quantification

P-1030 MethylFlash™ Global DNA Methylation (5-mC) ELISA Easy Kit (Colorimetric)
P-1032 MethylFlash Global DNA Hydroxymethylation (5-hmC) ELISA Easy Kit (Colorimetric)

Hydroxymethylated DNA Immunoprecipitation

P-1038 MethylFlash™ Hydroxymethylated DNA Immunoprecipitation (hmeDIP) Kit

DNA/RNA Damage and Repair

OP-0001 EpiQuik™ Superoxide Dismutase Activity/Inhibition Assay Kit (Colorimetric)
OP-0005 CytoX-Violet Cell Proliferation/Cytotoxicity 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-6008 EpiQuik™ 8-OhG RNA Damage Quantification Direct Kit (Colorimetric) new product
P-6001 EpiQuik™ In Situ DNA Damage Assay Kit
P-6005 EpiQuik™ In-Situ and Ex-Situ Hydrogen Peroxide (H2O2) Assay Kit new product

DNA Damage and Repair Antibodies

(See http://www.epigentek.com/catalog/dna-damage-repair-c-35_70.html)