8-OHdG-EIA Kit

Quantitative Assay for 8-Hydroxy-2'-deoxyguanosine

Catalog Number: A21026 (AOXRE Catalog Number 21026)

For Research Use Only. Not For Use in Diagnostic Procedures.

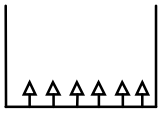
READ ENTIRE INSERT BEFORE BEGINNING ASSAYS!

Store at 2 to 8°C.

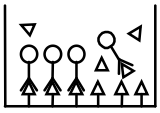
SUMMARY OF THE TEST

The 8-OHdG-EIA Kit is a competitive enzyme-linked immunosorbent assay (ELISA) for quantitative measurement of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in tissue, serum, plasma and urine resulting from oxidative damage to DNA. 8-OHdG is produced during DNA repair and its measurement may be useful as a marker of oxidative damage due to aging, cancer and other degenerative diseases.

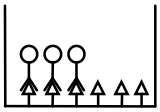
PRINCIPLES OF THE PROCEDURE



1. The 8-OHdG monoclonal antibody and the sample or standard are added to a microtiter plate well which has been precoated with 8-OHdG. The 8-OHdG in the sample or standard competes with the 8-OHdG bound on the plate for the 8-OHdG monoclonal antibody binding sites. Therefore, higher concentrations of 8-OHdG in the sample solution lead to a reduced binding of the antibody to the 8-OHdG on the plate.

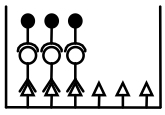


2. The antibodies that are bound to the 8-OHdG in the sample are washed out of the well, while those that have bound to the 8-OHdG coated on the plate will remain.



3. The enzyme-labeled secondary antibody is added and binds to the monoclonal antibody that remains on the plate.

4. Unbound enzyme-labeled secondary antibody is removed by a wash step.



5. Addition of a chromogen results in the development of color in proportion to the amount of antibody bound to the plate.

6. The color reaction is terminated and the absorbance is measured.

REAGENTS

The 8-OHdG-EIA Kit is sufficient for 18 sample assays in triplicate, and consists of the following reagents:

8-OHdG Microtiter Plate	Precoated With 8-OHdG (8×12 wells, Split Type) 1 Plate
Primary Antibody	Monoclonal Antibody Specific For 8-OHdG 1 Vial
Primary Antibody Dilution Buffer	Phosphate Buffered Saline 6 mL
Secondary Antibody	HRP-Conjugated Antibody 1 Vial
Secondary Antibody Dilution Buffer	Phosphate Buffered Saline 12 mL

Chromogen	3,3',5,5'-Tetramethylbenzidine 250 µL
Chromogen Dilution Buffer	Hydrogen Peroxide/Citrate-Phosphate Buffered Saline 12 mL
Washing Buffer (5x)	Concentrated Phosphate Buffered Saline 2 at 26 mL
Stop Solution	1M Phosphoric Acid 12 mL
Standards 1-6	0.5, 2.0, 8.0, 20.0, 80.0, 200.0 ng/mL 8-OHdG (1 vial Each) 1 mL each
Plate Seal	Adhesive sheet for covering plate 1 Sheet

Warnings and Precautions

For Research Use Only. Not For Use in Diagnostic Procedures.
For *In Vitro* Use Only.
Use established laboratory precautions when handling or disposing any chemicals contained in this product. Refer to the Material Safety Data Sheet for risk, hazard and safety information.

Preparation

Dilute *Washing Buffer* 1/5 with deionized water prior to use.

Reagent Storage and Stability

Store reagents tightly closed at 2-8°C in the dark. **Do not freeze.** When stored properly, the unopened reagents are stable until the expiration date printed on the box label.

SAMPLE GUIDELINES

- Urine: Pre-treatment of clear samples is not needed. Centrifugation at 2,000 x g for 10 minutes is recommended for samples containing precipitate.
- Serum: Filtration of serum using ultra-filtration (cut off molecular weight 10,000) is necessary.
- DNA in Tissue: Extract and digest DNA in the samples before adding to assay.

PROCEDURE

Instrument

Microtiter Plate Reader set up to measure absorbance at 450 nm.

Materials Required But Not Provided

- 50 µL micropipettor and tips
- Multi-channel (50-200 µL) micropipettor or repeating pipettor and tips
- Trays for multi-channel micropipettor
- 37ºC Incubator or microtiter plate shaker/incubator (preferred).

Quality Control Procedures and Materials

If controls are desired, aliquot pooled samples and freeze at -70°C. Establish acceptable assay ranges for these controls based on in-house mean and precision.

Assay Method

Bring all reagents and samples to room temperature before use.

1. Reconstitute the *Primary Antibody* with the *Primary Antibody Dilution Buffer*.
2. Add 50 µL of sample or standard per well. To prevent edge effects, do not use outermost rows (Rows A and H).
3. Add 50 µL of reconstituted *Primary Antibody* to all wells except Blank. Seal plate tightly with *Plate Seal*. Shake plate from side to side to mix fully. Incubate at 37ºC for 1 hour.

4. Pour off contents of plate. Pipette 250 µL diluted *Washing Buffer* into each well. Wash thoroughly by agitation, dispose of *Washing Buffer*. Invert plate and blot against clean paper towel to remove any remaining washing buffer. Repeat wash twice.
5. Reconstitute the *Secondary Antibody* with the *Secondary Antibody Dilution Buffer*.
6. Add 100 µL of reconstituted *Secondary Antibody* per well. Seal plate tightly with *Plate Seal*. Shake plate from side to side to mix fully. Incubate at 37ºC for 1 hour.
7. Dilute the *Chromogen* with 100 volumes of *Chromogen Dilution Buffer*.
8. Repeat step 4.
9. Add 100 µL of the diluted *Chromogen* per well. Shake plate from side to side to mix fully. Incubate at room temperature in the dark for 15 minutes.
10. Add 100 µL of the *Stop Solution*, mix, wait 3 minutes and read the absorbance at 450 nm.

A Typical Layout for Assaying Triplicates of Each Sample.

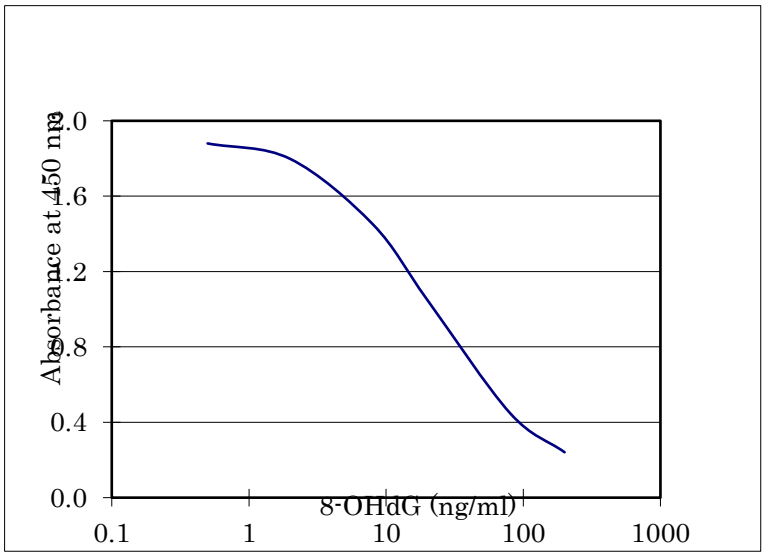
	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank (x3)			X	X	X	X	X	X	X	X	X
B	0.5 ng/mL			1		(x3)		7	(x3)		13	(x3)
C	2.0 ng/mL			2				8			14	
D	8.0 ng/mL			3				9			15	
E	20.0 ng/mL			4				10			16	
F	80.0 ng/mL			5				11			17	
G	200.0 ng/mL			6				12			18	
H	X	X	X	X	X	X	X	X	X	X	X	X

Upper and lower wells (rows A and H) are not used due to edge effects.

RESULTS

Details of Calibration

Generate the standard curve by plotting absorbance vs. log of concentration. The recommended curve fit analysis is the 4-parameter logistic function: $y = \frac{a-d}{1+(x/c)^b} + d$



Calculations

Use the absorbance values obtained for test samples to determine concentration from the calibration curve.

LIMITATIONS OF THE PROCEDURE

To avoid edge effects, the use of outermost wells (Rows A and H) is not recommended. To maintain uniform temperature across the plate, fill any unused wells with water to the same volume as sample wells during incubation steps.

REFERENCES

1. R.G. Cutler. *Am.J.Clin.Nutr*. **50:**373S-379S, 1991.
2. H. Kasai, P.F. Crain, Y. Kuchino, S. Nishimura, A. Ootsuyama and H. Tanooka. *Carcinogenesis* **7:**1849-51, 1986.
3. C.G. Fraga, M.K. Shigenaga, J.W. Park, P. Degan and B.N. Ames. *Proc.Natl.Acad.Sci.USA* **87:**4533-4537, 1990.
4. M.K. Shigenaga and B.N. Ames. *Free Radical Biology and Medicine* **10:**211-6, 1991.
5. S. Okamoto,and H. Ochi. *Chemical Abst.* 129859a, 1992.
6. S.S. Kantha, S. Wada, H. Tanaka, M. Takeuchi, S. Watabe and H. Ochi. *Biochem.Biophys.Res.Comm.* **223:**278-82, 1996.
7. S. Toyokuni, T. Tanaka, Y. Hattori, Y. Nishiyama, A. Yoshida, K. Uchida, H. Hiai, H. Ochi and T.Osawa. *Lab.Invest.* **76:**365-74, 1997.
8. M. Erhola, S. Toyokuni, K. Okada, T. Tanaka, H. Hiai, H. Ochi, K. Uchida, T. Osawa, M.M. Nieminen, H. Alho and P.K. Lehtinen. *FEBS Letters* **40:**287-91, 1997.
9. H. Ochi. *Rouka seigyo syokuhin no kaihatsu* (Development of foods for controlling aging) Korin Press, p. 294, 1995.

*Oxis*Research™

2946 Scott Blvd

Santa Clara, CA 95054, U.S.A.

E-mail: sales@lumiquick.com

Telephone: 408-855-0061

www.lumiquick.com, www.aoxre.com

Made in the U.S.A.

BIOXYTECH® 8-OHdG-EIA Procedure Flow Chart