



OXIDATIVE STRESS BIOMARKERS  
FOR THE ADVANCEMENT OF BASIC AND  
APPLIED RESEARCH RELATED TO AGING

## **Sample Pretreatment Procedure for 8-OHdG Detection**

**(For use with High Sensitive 8-OHdG ELISA Kit – *KOG-HS10E*)**

To assay properly, please pre-treat samples as described below. In addition, please avoid repeated freeze thaw samples.

### **1. Urine Sample:**

If the urine sample is clear, pretreatment is not recommended. Centrifugation at 2,000~5,000G for 10 ~15 minutes is recommended for opaque samples only.

### **2. Serum Sample:**

Blood samples drawn must be processed to separate the clear serum immediately. Further, in order to remove all interfering substances, filtration of the clear serum using an ultra filter (cut off molecular weight 10,000) is necessary.

Prepare the ultra filter following the manufacture's protocols.

### **3. DNA Sample from organs, tissues, and cultured cells:**

It is necessary to extract samples and digest DNA before using this kit.

#### ***1. Extract DNA from cells.***

Extract DNA from the organ, tissue, or the cell homogenates using a DNA extraction kit. Please follow the manufacture's manual. As an example for DNA extraction kit, DNA Extractor WB Kit (Sodium Iodide Method, WAKO Pure Chemical Industries, Ltd., Japan) is suitable.

#### ***2. Calculation of DNA Concentration and Purity.***

- a) DNA Solution is diluted to 20-50 micro g/mL with TE buffer.
- b) Measure absorbance at wavelength 230nm, 260nm, 280nm and 320nm.
- c) Calculate DNA Concentration. (1.0 of Absorbance at 260nm is equal to 50 micro g/mL DNA)

DNA concentration (micro g/mL) = (Absorbance at 260nm) \* (50 micro g/mL)  
\* dilution rate

d) Check the purity of DNA sample.

The ratio of (Absorbance at 260nm) : (Absorbance at 280nm) is typically between 1.8 to 1.85.

The purity of isolated DNA is assessed by the ratio of (Absorbance at 260nm) : (Absorbance at 230nm), and should be between 2.2 to 2.25.

### ***3. Enzymatic Digestion of DNA.***

a) Dissolve 200 micro gram of extracted DNA in 135 micro L of water.

b) Add 15 micro L of 200mM sodium acetate and 6 units of nuclease P1 (15 micro L, 1 mg/mL) to the DNA Solution, and then incubate for 30 min to 1hr at 37 degree C after Argon substitution.

c) Add 1M Tris-HCl buffer (15 micro L, pH 7.4) and 2 units of alkaline phosphatase (7 micro L, 200 units/0.7mL), and incubate for 30 min to 1hr at 37 degree C after Argon substitution.

d) To remove enzymes and other macromolecules, the hydrolysates are filtered through Millipore Microcon YM-10(catalog#42407) at 14000rpm for 10min.

e) Apply 50 micro L of DNA digest to the wells of ELISA kit. It is recommended that analysis procedure mentioned here should be completed in one day.

### ***4. Reagents.***

a) Water: Purified Water.

b) TE buffer: 1mM EDTA in 10mM Tris-HCl(pH8.0)