GENTAUR

DATA SHEET

IM-KOGNW 050915E

FOR RESEARCH USE ONLY

Catalog No. N213100

"New 8-OHdG Check" INSTRUCTIONS

The 8-OHdG Check is a competitive *in vitro* enzyme-linked immunosorbent assay (ELISA) for quantitative detection of the oxidative DNA adduct 8-hydroxy-2'-deoxyguanosine (8-OHdG) . Suitable for urine, serum, plasma, tissue and other biological samples. For research use only. Not for diagnostic or medical use. Read entire insert before use.

1. Kit Contents

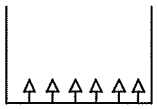
①8-OHdG Microtiter Plate	: Precoated with 8-OHdG(8×12wells, split type)	1 plate
②Primary Antibody	: Anti 8-OHdG Monoclonal antibody (clone N45.1)	1 vial
③Primary Antibody Solution	: Phosphate buffered saline	1 vial (6mL)
④Secondary Antibody	: HRP-conjugated anti mouse antibody	1 vial
⑤Secondary Antibody Solution	: Phosphate buffered saline	1 vial (12mL)
⑥Chromatic Solution	: 3,3',5,5'-tetramethylbenzidine	1 vial (0.25mL)
⑦Diluting Solution	: Hydrogen peroxide/citrate-phosphate buffered saline	1 vial (12mL)
⑧Washing Solution(5x)	: 5 times concentrated phosphate buffered saline	2 vials (26mLx2)
⑨Reaction Terminating Solution	: 1M Phosphoric acid	1 vial (12mL)
⑩Standard 8-OHdG Solution	: Purified 8-OHdG (0.5, 2, 8, 20, 80, 200 ng/mL)	1 vial each
⑪Plate Seal		2 sheets

All reagent should be stored at 2-8°C. The expiry date is 12 months after manufacture.
After the seal of above contents are opened, this kit should be used within 2 weeks.

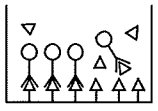
2. Additional Materials and Equipments Required

①Distilled water.
②50µL micropipettor and pipette tips
③8-channel micropipettor (50～200µL) and pipette tips
④Reagent trays for 8-channel micropipettor
⑤A 37°C incubator
⑥Microtiter plate reader (measuring wavelength = 450 nm)

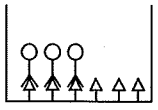
3. Priciples of the Procedure



①The anti-8-OHdG monoclonal antibody and the sample or standard are added to the microtiter plate which has been precoated with 8-OHdG. The 8-OHdG monoclonal antibody reacts competitively with the 8-OHdG bound on the plate and the 8-OHdG in samples solution. 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.

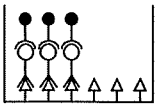


②The antibodies which are bound to the 8-OHdG in the sample are washed away from the antibodies that have bound to the 8-OHdG coated on the plate.



③An enzyme-labeled secondary antibody, which is added to the plate, binds to the monoclonal antibody which is bound to the 8-OHdG coated on the plate.

④Unbound HRP-conjugated secondary antibody is removed by washing.



⑤Addition of the substrate solution results in the development of color in proportion to the amount of anti 8-OHdG antibody bound to the plate.

⑥The reaction is terminated by phosphoric acid, and absorbance at 450 nm is measured.

4. Assay Procedure

Bring all reagents and samples to room temperature (20-25°C) before use.

A) Reconstitute the *②Primary Antibody* with the *③Primary Antibody Solution.*

B) Add 50µL of sample or *⑩Standard* per well, as shown in plate diagram.

C) Add 50µL of reconstituted primary antibody per well. Shake the plate from side to side and mix fully. Cover plate with adhesive strip, making sure it is sealed tightly. Incubate at 37°C for 1 hour.

D) Mix 1 volume of *⑧Washing solution(5x)* with 4 volumes of distilled water.

E) Pour off contents of wells into sink. Pipette 250µL washing solution into each well. After washing thoroughly by shaking the plate from side to side, dispose of washing solution. Invert plate and blot against clean paper towel to remove any remaining washing buffer. Repeat wash two times more. The use of washing machines or aspirators is not recommended.

F) Reconstitute the *④Secondary Antibody* with the *⑤Secondary Antibody Solution.*

G) Add 100µL of constituted secondary antibody per well. Shake the plate from side to side and mix fully. Cover the plate with an adhesive strip. Incubate 37°C for 1 hour.

H) At the end of the incubation period, repeat washing as in step E.

I) Prepare substrate solution. Add 1 volume of *⑥Chromatic Solution* to 100 volumes of *⑦Diluting Solution* just before use. Add 100µL of substrate solution per well. Shake the plate from side to side and mix fully. Incubate at room temperature for 15 minutes in the dark.

J) Add 100µL of the *⑨Reaction Terminating Solution.* Shake the plate from side to side and mix fully.

K) Measure the absorbance at 450 nm using mictotiter plate reader.
Use a standard curve to determine the amount of 8-OHdG present in test samples. Generate the standard curve by plotting absorbance vs. log (concentration of standards). Then use the absorbance values obtained for the test samples to determine the concentrations.

Notices

1) Sample Pretreatment
To assay properly, please pre-treat samples as follows. Avoid repeated freeze and thaw.
   ① Urine : If it's clear, pretreatment is not needed.
   Centrifugation at 2,000 ~ 5,000g for 10 ~ 15 minutes is recommended for opaque samples only.
   ② Serum : Blood samples must be separated to serum immediately. To separate interfering substances, filtration of serum using an ultra filter (cut off molecular weight 10,000) is necessary. Pre-treat ultra filter following to the maker's manuals. In order to reduce deviation, diluting samples by more than twice, while paying attention to concentration range is suggested.
   ③ DNA in Tissue : It's necessary to extract and digest DNA in the samples beforehand.

2) Measurement
   ① Strict Control of Incubation Temperature
   Measured values may be very much affected with the incubation temperatures, particularly during primary antibody reaction period. Please pay attention to followings;
      a. Try to keep uniform temperature inside a plate constantly.
      b. It's recommendable to use water bath rather than dry incubators for the incubation.
   ② Adjustment of pH for samples
   It is necessary to maintain pH of a sample mixed with primary solution between 6.0 to 8.0. It's recommendable for abnormal urine samples to be diluted with PBS by three times.
   ③ Thoroughly Washing of Micro plates
   It's recommendable to throw the micro plate down on clean paper towel to remove solution in side wells, after a plate is turn over and solution in side wells is discarded.
   ④ Cleaning of Instruments and Vessels
   Instruments and vessels (such as tips, trays for 8 channel pipettor) to be used, must be clean. If such tools are used repeatedly, please boil or steep them into alkaline cleanser, then wash thoroughly and dry them before use.

3) Split Usage
   ① Remained parts of kit (plate and reagents) must be kept in a refrigerator and must be used within two weeks after opened.
   ② Plates and reagents except chromatic solution are taken out from refrigerator and are kept in room temperature beforehand. Necessary volume of Chromatic solution may be added to adequate volume only of Diluting solution just before the reaction. Keep it in the dark.

4) Use of wells

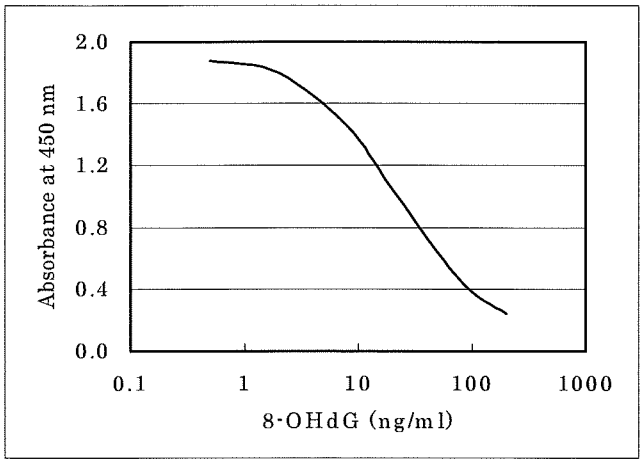
① To avoid edge effects, the use of outer most wells is not recommended. To maintain the uniform temperature within the wells, please full same volume of solutions or water to the unused wells.

② Blank wells : at the operation of step C, those wells which are not added the reconstituted primary antibody, will serve as blank wells.

③ The figure below shows a typical layout for sample loading in triplicates for each sample. Wells indicated with a cross-mark (×) in A and H lines are not used. With this layout, a maximum of 18 samples can be assayed in a plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank (×3)			×	×	×	×	×	×	×	×	×
B	Standard 0.5 ng/mL			Sample-1			Sample-7			Sample-13		
C	Standard 2 ng/mL			Sample-2			Sample-8			Sample-14		
D	Standard 8 ng/mL			Sample-3			Sample-9			Sample-15		
E	Standard 20 ng/mL			Sample-4			Sample-10			Sample-16		
F	Standard 80 ng/mL			Sample-5			Sample-11			Sample-17		
G	Standard 200ng/mL			Sample-6			Sample-12			Sample-18		
H	×	×	×	×	×	×	×	×	×	×	×	×

5. Standard Curve



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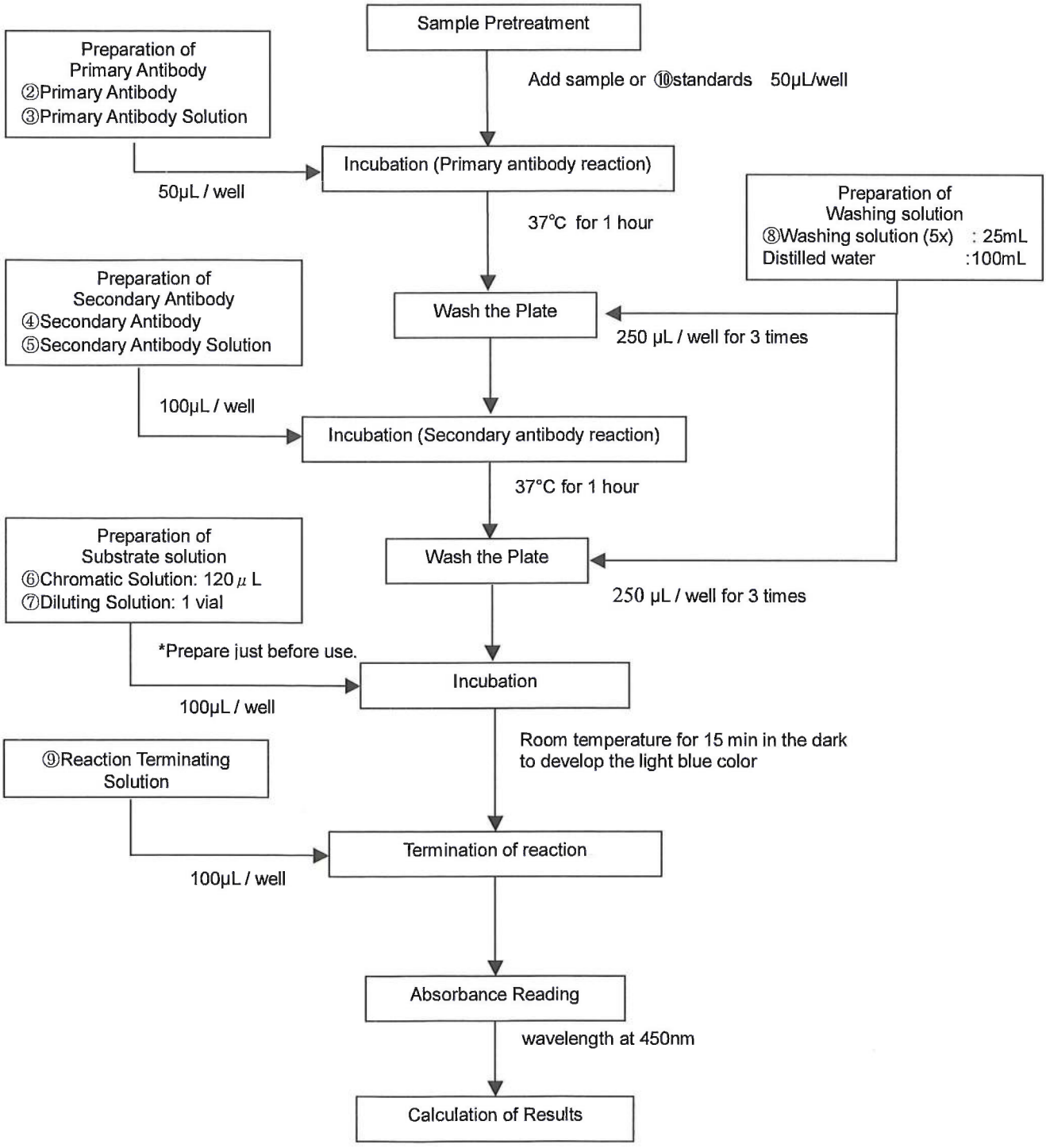
7. Assay Flowchart



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