KAMIYA BIOMEDICAL COMPANY

8-OHdG ELISA

For the quantitative determination of 8-OHdG in urine, serum, plasma, tissue and other biological samples.

Cat. No. KT-447

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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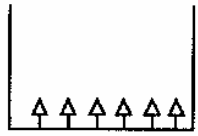
PRODUCT

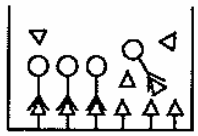
The **K-*ASSAY*®** 8-OHdG ELISA is a competitive *in vitro* enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of the oxidative DNA adduct 8-hydroxy-2'-deoxyguanosine (8-OHdG) in urine, serum, plasma, tissue, and other biological samples.

PRINCIPLE

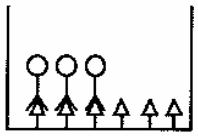
1. The anti-8-OHdG monoclonal antibody and the sample or calibrator are added to the microtiter plate which has been pre-coated with 8-OHdG. The 8-OHdG monoclonal antibody reacts competitively with the 8-OHdG bound on the plate and the 8-OHdG in sample solution. Therefore higher concentrations of 8-OHdG in the sample solution lead to a reduced binding of the antibody to the 8-OHdG on the plate.



2. The 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.

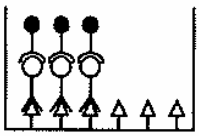


3. An enzyme-labeled secondary antibody, which is added to the plate, binds to the monoclonal antibody which is bound to the 8-OHdG coated on the plate.



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

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



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

COMPONENTS

- Pre-coated 8-OHdG Microtiter Plate: 96-wells (split type)
- Primary Antibody: Anti 8-OHdG monoclonal antibody
- Primary Antibody solution: Phosphate buffered saline, 6 mL
- Secondary Antibody: HRP-conjugated anti mouse antibody
- Secondary Antibody solution: Phosphate buffered saline, 12 mL
- Chromatic Solution: 3,3',5,5'-tetramethylbenzidine, 0.25 mL
- Diluting Solution: Hydrogen peroxide/citrate-phosphate buffered saline, 12 mL
- Washing Solution (5X): concentrated phosphate buffered saline, 2 x 26 mL
- Stop Solution: 1M Phosphoric acid, 12 mL
- 8-OHdG Calibrator Solution: Purified 8-OHdG (0.5, 2, 8, 20, 80, 200 ng/mL)
- Plate Seal: 2 sheets

Materials or Equipment required but not provided

- Distilled water
- 50 µL micropipettor and pipette tips
- 8-channel micropipettor (50-200 µL) and pipette tips
- Reagent trays for 8-channel micropipettor
- A 37℃ incubator
- Microtiter plate reader (measuring wavelength = 450 nm)

PREPARATION OF REAGENTS

A. Test Sample

1. Avoid freezing and thawing of samples.
2. *Urine*: If it's clear, pre-treatment is not necessary. Otherwise, centrifugation at 2,000-5,000 g for 10-15 mins is recommended for opaque samples only.
3. *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 the maker's manual. In order to reduce deviation, diluting samples by more than 2 times, while paying attention to concentration range is recommended.
4. *DNA in tissue*: Extraction and digestion of DNA in samples beforehand is necessary.

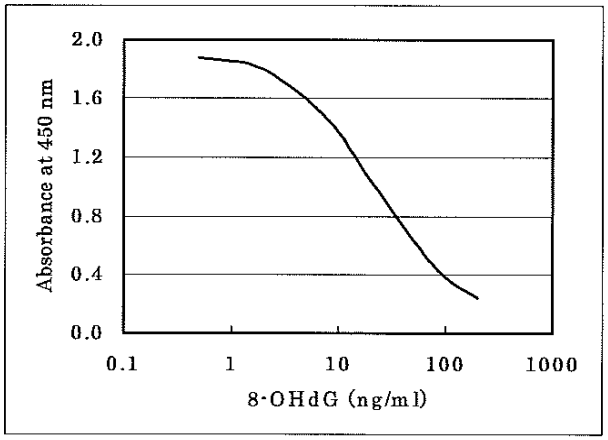
PROCEDURE

1. Bring all reagents and samples to room temperature (20-25℃) before use.
2. Reconstitute the *Primary Antibody* with the *Primary Antibody Solution.*
3. Add 50 µL of sample or calibrator per well.
4. Add 50 µL of reconstituted primary antibody per well. Shake the plate from side to side and mix fully. Cover the plate with adhesive strip, making sure it is sealed tightly. Incubate at 37℃ for 1 hour.
5. Mix 1 volume of washing solution (5X) with 4 volumes of distilled water.
6. Pour off contents of wells into sink. Pipette 250 µL of 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 2 more times. (The use of washing machines or aspirators is not recommended)
7. Reconstitute the *Secondary Antibody* with the *Secondary Antibody Solution*.
8. Add 100 µL 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℃ for 1 hour.
9. At the end of the incubation period, repeat washing as in step 6.
10. Prepare substrate solution. Add 1 volume of the *Chromatic Solution* to 100 volumes of *Diluting Solution* just before use. Add 100 µL of substrate solution per well. Shake the plate from side to side and mix fully. Incubate at room temperature for 15 mins in the dark.
11. Add 100 µL of the *Reaction Terminating Solution*. Shake the plate from side to side and mix fully.
12. Measure the absorbance at 450 nm using the microtiter plate reader.

CALCULATION OF RESULTS

1. Use a calibration curve to determine the amount of 8-OHdG present in test samples.
2. Generate the calibration curve by plotting absorbance versus log (concentration of calibrators).
3. Use the absorbance values obtained from the test samples to determine the concentrations.

A typical calibration curve is shown below. Do not use this curve to determine results.



TECHNICAL HINTS

1. Measurement

- Strict control of incubation temperature: measured values may be very much affected by the incubation temperatures, particularly during primary antibody reaction period. Pay close attention to the following:
  a) Try to keep uniform temperature inside plate constantly.
  b) It is recommended to use water baths for incubation instead of dry incubators.
- 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 is recommended to dilute abnormal urine samples with PBS 3 times.
- Thorough washing of micro plates: It is recommended to place the micro plate down on clean paper towel to remove solution inside wells. Discard solution inside wells.
- Cleaning of instruments: instruments and vessels (such as tips, trays, 8-channel pipettor) to be used must be clean. If such tools are used repeatedly, boil or steep them into an alkaline cleanser, then wash thoroughly and dry them before use.

2. Split Usage

- Remaining parts of the kit (plate and reagent) must be kept in a refrigerator and must be used within 2 weeks after being opened.
- Plates and reagents except the chromatic solution are taken out of the refrigerator and should be kept in room temperature beforehand. The necessary volume of the chromatic solution may be added to an adequate volume only of diluting solution just before the reaction. Keep it in the dark.

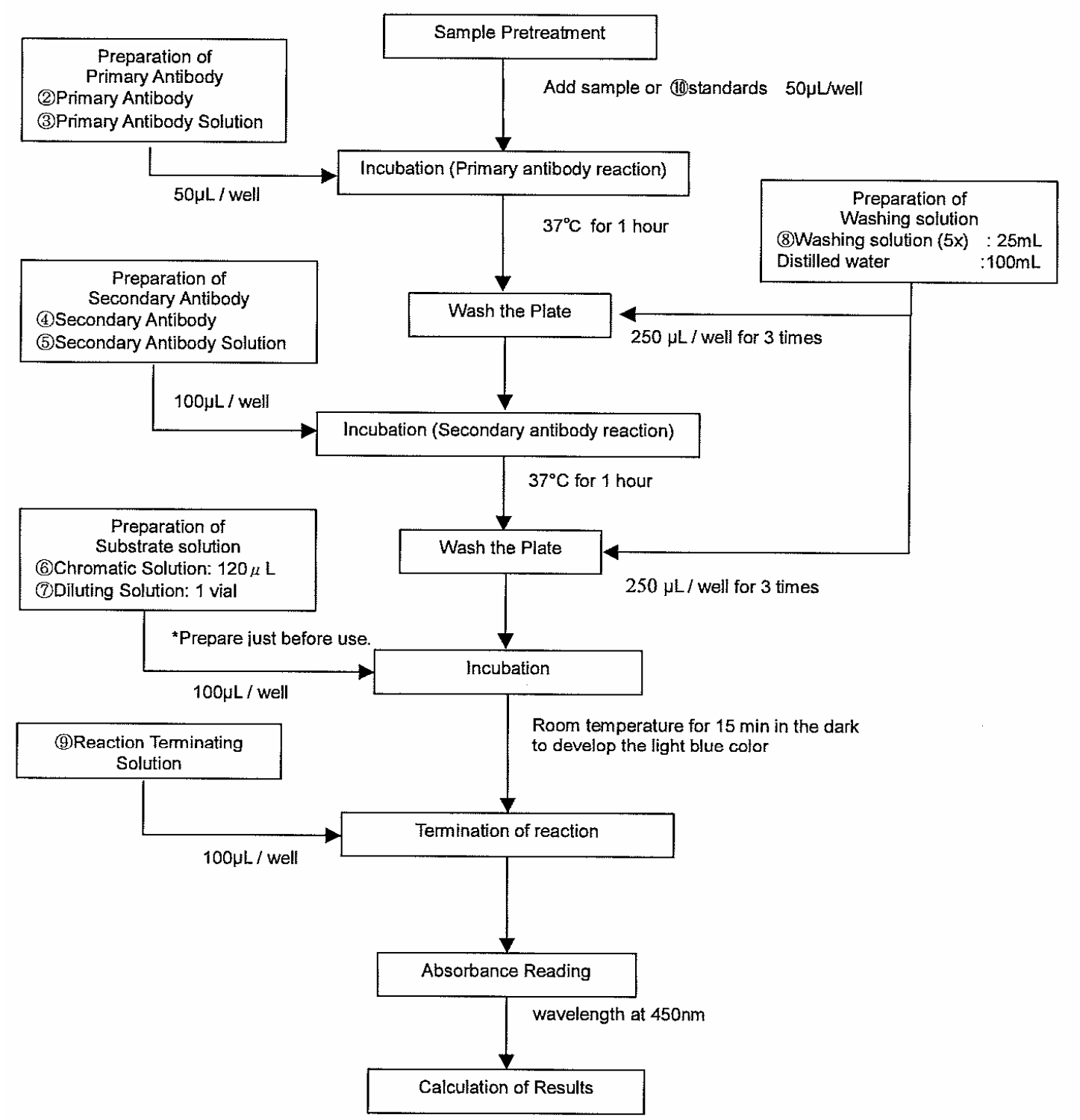
3. Use of Wells

- To avoid edge effects, the use of outer most wells is not recommended. To maintain the uniform temperature within the wells, please fill same volume of solutions or water to the unused wells.
- Wells that are not filled with reconstituted primary antibody will serve as blank wells.
- The figure below shows a typical layout for sample loading in triplicates for each sample. Wells indicated with an “x” in rows “A” and “H” 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	×	×	×	×	×	×	×	×	×	×	×	×

STORAGE

Store at 4°C until the expiration date. After opening, the kit should be used within 2 weeks.



FOR RESEARCH USE ONLY

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