"Highly Sensitive 8-OHdG Check" INSTRUCTIONS

The 8-OHdG ELISA kit is a competitive *in vitro* enzyme-linked immunosorbent assay for quantitative measurement of the oxidative DNA adduct 8-hydroxy-2'-deoxyguanosine (8-OHdG) in tissue, urine etc.

1. Kit Contents

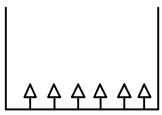
①8-OHdG Microtiter plate	: precoated with 8-OHdG (8×12wells, split type)	1 plate
②Primary antibody	: monoclonal antibody specific for 8-OHdG	1 vial
③Primary antibody solution	: phosphate buffered saline	1 vial (ca.6ml)
④Secondary antibody	: HRP-conjugated antibody	1 vial
⑤Secondary antibody solution	: phosphate buffered saline	1 vial (ca.12ml)
⑥Chromatic solution	: 3,3',5,5'-tetramethylbenzidine	1 vial (ca.0.25ml)
⑦Diluting solution	: hydrogen peroxide/citrate-phosphate buffered saline	1 vial (ca.12ml)
⑧Washing solution(×5)	: 5 times concentrated phosphate buffered saline**	2 vials (ca.26ml×2)
⑨Reaction terminating solution	: 1M phosphoric acid	1 vial (ca.12ml)
⑩Standard 8-OHdG solution	: 0.125, 0.25, 0.5, 1, 4, 10 ng/ml 1vial each	total 6 vials (ca.1ml)
⑪Plate seal		2 sheets

*All reagent should be refrigerated at 2-8℃.
*The kit should not be used beyond 9 months past the manufacturers date stamped on the exterior of the box.
**Dilute ⑧Washing solution by 5 times (v/v) with distilled water for use.

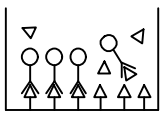
2. Additional Materials and Equipments Required

①50µl pipettor and tips
②8-channel micropipettor and tips (50-200µl)
③Trays for 8-channel pipettor
④Refrigerator
⑤Microplate reader (450 nm)

3. Summary of Assay Procedure

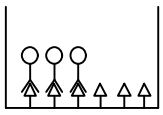


①The 8-OHdG monoclonal antibody and the sample or standard are added to the microtiter plate which has been precoated with 8-OHdG. The 8-OHdG monoclonal antibody reacts competitively with the 8-OHdG bound on the plate and the 8-OHdG in samples solution. Therefore higher concentrations of 8-OHdG in the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



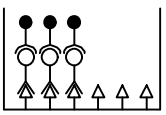
②The antibodies which are bound to the 8-OHdG in the sample are washed away from the antibodies that have bound to the 8-OHdG coated on the plate.

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

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



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

4. Assay Procedure

Bring all reagents and samples to room temperature before use (20-25℃), and proceed through the following 10 steps.

① Reconstitute the primary antibody with the primary antibody solution. Allow dissolving completely.

② Add 50µl of sample or standard per well, as shown in plate diagram. To ensure accuracy, do not use outer most wells.

③ 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 4℃ for over night.

④ Pour off contents of plate 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.

⑤ Reconstitute the secondary antibody with the secondary antibody solution. Allow dissolving completely.

⑥ 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 room temperature for 1 hour.

⑦ At the end of the incubation period, repeat wash as in step ④.

⑧ Reconstitute the chromatic solution (enzyme substrate solution) with 100 times volume of the diluting solution. Add 100µl of the reconstituted enzyme substrate per well. Shake the plate from side to side and mix fully. Incubate at room temperature for 15 minutes. This incubation should be done in the dark, i.e. shield the plate with aluminum foil.

⑨ Add 100µl of the reaction terminating solution. Shake the plate from side to side and mix fully.

⑩ After terminating the reaction, read the absorbance at 450 nm.
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 desired bellow. In addition, please avoid repeated freeze thaw samples. In order to confirm the aptitude of assay methods on a new sample, implementing recovery test of standard 8-OHdG added into the new sample is recommended.
   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 much affected by the reacting conditions. Please pay attention to the reacting conditions.
   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 piled 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 (⑥) 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 (⑦) just before the reaction. Keep it in the dark.

4) Wells Usage

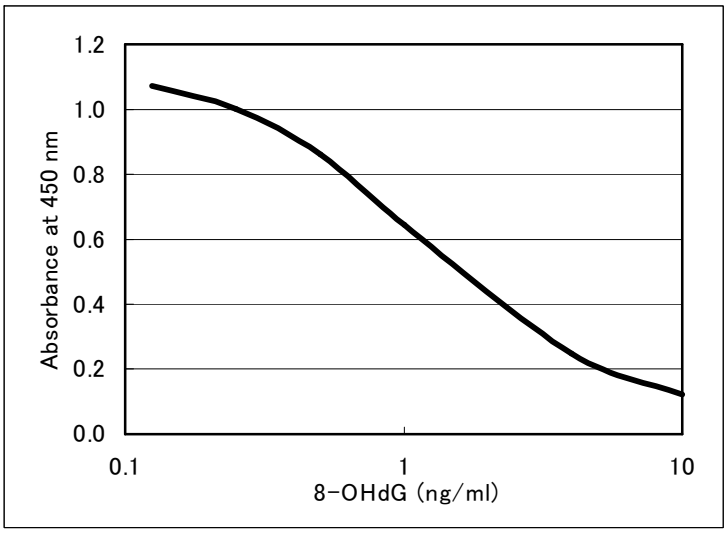
① To avoid edge effects, the use of outer most wells is not recommended. To maintain the uniform temperature within the wells, please full same volume of solutions or water to the unused wells.

② Blank wells : at the operation of step ③, 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	0.125ng/ml(×3)				1	(×3)		7	(×3)		13	(×3)
C	0.25ng/ml(〃)				2	(〃)		8	(〃)		14	(〃)
D	0.5ng/ml(〃)				3	(〃)		9	(〃)		15	(〃)
E	1ng/ml(〃)				4	(〃)		10	(〃)		16	(〃)
F	4ng/ml(〃)				5	(〃)		11	(〃)		17	(〃)
G	10ng/ml(〃)				6	(〃)		12	(〃)		18	(〃)
H	×	×	×	×	×	×	×	×	×	×	×	×

5. Standard Curve



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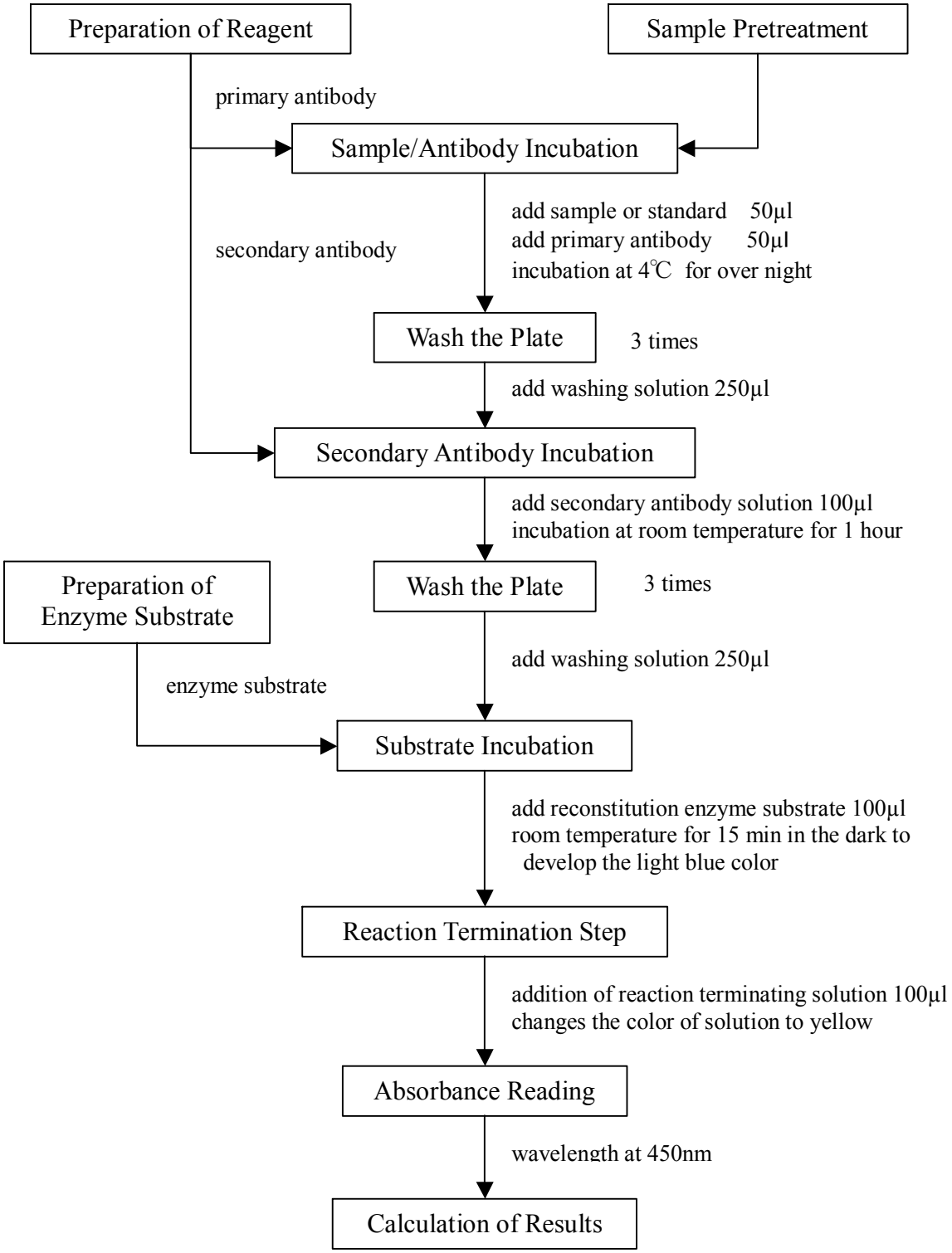
7. Assay Flowchart



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