8-hydroxy-2′-deoxyguanosine (8-OHdG) ELISA

Catalog Number: HDG39-K01
96 Wells
For Research Use Only
v. 1.0

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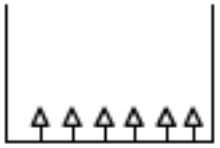
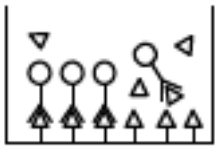
Intended Use:

The Eagle Biosciences 8-hydroxy-2'-deoxyguanosine (8-OHdG) ELISA assay kit is intended for the quantitative determination of adduct 8-hydroxy-2′-deoxy-guanosine (8-OHdG) in urine, serum or biological samples by enzyme linked immunoassay (ELISA). The 8-hydroxy-2'-deoxyguanosine (8-OHdG) ELISA assay kit is for research use only and not to be used in diagnostic procedures.

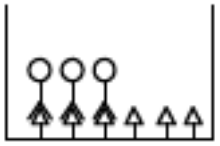
Principle of Procedure:



1. The 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.

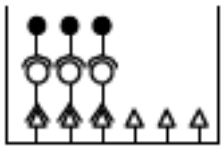


2. The antibodies which are bound to the 8-OHdG in the sample are washed away from the antibodies that have bound to the 8-OHdG coated on the plate.



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 phosphoric acid, and absorbance at 450 nm is measured.

Materials Provided:

1. 8-OHdG Microtiter Plate, Precoated with 8-OHdG (8 × 12wells, split type): 1 plate
2. Primary Antibody, Anti 8-OHdG Monoclonal antibody (clone N45.1): 1 vial
3. Primary Antibody Solution, Phosphate buffered saline: 1 vial (6mL)
4. Secondary Antibody, HRP-conjugated anti mouse antibody: 1 vial
5. Secondary Antibody Solution, Phosphate buffered saline: 1 vial (12mL)
6. Chromatic Solution, 3,3',5,5'-tetramethylbenzidine: 1 vial (0.25mL)
7. Diluting Solution, Hydrogen peroxide/citrate-phosphate buffered saline:

1 vial (12mL)
8. Washing Solution(5x), 5 times concentrated phosphate buffered saline: 2 vials (26mL x 2)
9. Reaction Terminating Solution, 1M Phosphoric acid: 1 vial (12mL)
10. Standard 8-OHdG Solution, Purified 8-OHdG (0.5, 2, 8, 20, 80, 200 ng/mL): 1 vial each
11. Plate Seal: 2 sheets

- All reagents of the 8-hydroxy-2'-deoxyguanosine (8-OHdG) ELISA assay 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.

Materials required but not provided

- Distilled water (Preparation of washing solution)
- 50 µL micropipettor and pipette tips
- 8-channel (50-200 µL) micropipettor and tips.
- Reagent trays for 8-channel micropipettor.
- 37 °C incubator.
- Microtiter plate reader (measuring wavelength 450 nm).

Sample Pretreatment

To assay properly with the 8-hydroxy-2'-deoxyguanosine (8-OHdG) ELISA assay, please pre-treat samples as follows. Avoid repeated freeze and thaw. It is necessary to maintain pH of a sample mixed with primary solution between 6.0 and 8.0.

- Urine:
  - If clear, pretreatment is not required.
  - Centrifugation at 2,000 ~ 5,000g for 10 ~ 15 minutes is recommended for opaque samples only.
  - It is recommended that abnormal urine samples be diluted with PBS up to three times.
- 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 manufacturer's manuals.
  - In order to reduce deviation, diluting samples by more than twice, while paying attention to concentration range is suggested.
- DNA in Tissue:
  - It is necessary to extract and digest DNA in the samples beforehand.

Assay Procedure:

Bring all reagents of the to room temperature before beginning 8-hydroxy-2'-deoxy-guanosine (8-OHdG) ELISA assay kit. Determine the number of microwells needed for the assay (each sample, standard, and control should be assayed in duplicate). Bring all reagents and samples to room temperature (20-25˚C) before use.

A) Reconstitute the *Primary Antibody* with the *Primary Antibody Solution*.
B) Add 50µL of sample or *Standard* per well.
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.

- Measured values may be very much affected with the incubation temperatures, particularly during primary antibody reaction period. It is recommended to use water bath rather than dry incubators for the incubation.

D) Mix 1 volume of *Washing 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 *Secondary Antibody* with the *Secondary Antibody Solution*.
G) Add 100µL of reconstituted 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 *Chromatic Solution* to 100 volumes of *Diluting 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 *Reaction Terminating Solution*. Shake the plate from side to side and mix fully.
K) Measure the absorbance at 450 nm using microtiter plate reader.

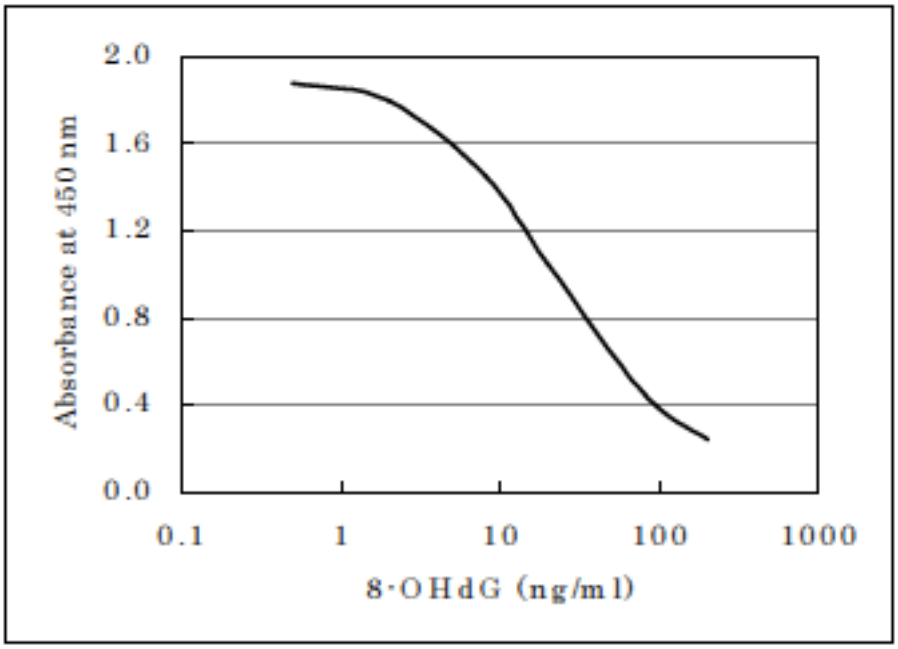
- Remained parts of kit (plate and reagents) must be kept in a refrigerator and must be used within two weeks after opening.

- 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.

Calculations:

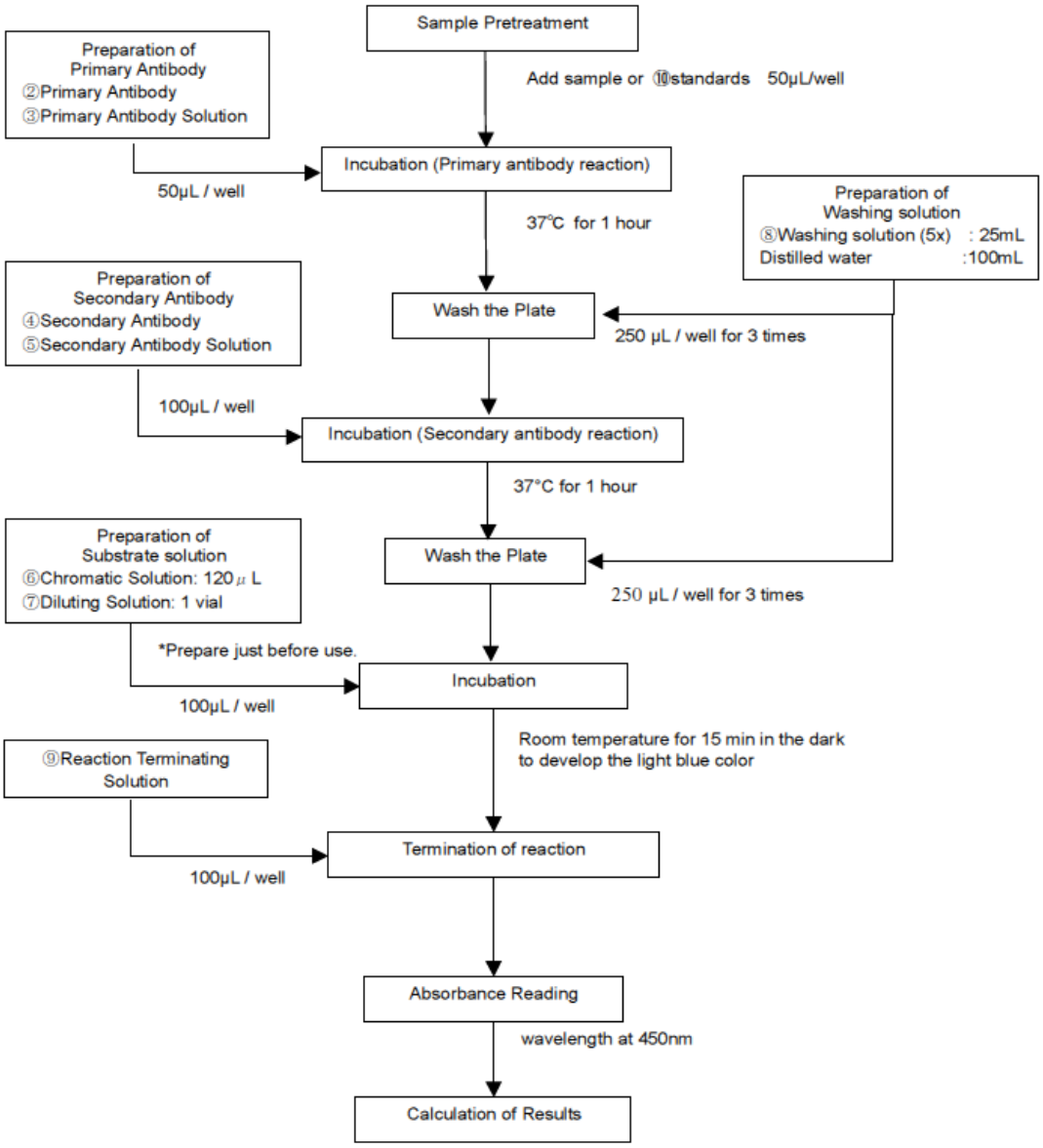
Generate the standard curve to determine the amount of 8-OHdG present in test samples. Generate the standard curve by plotting absorbance vs. log (concentration of standards). Then use the absorbance values obtained for the test samples to determine the concentrations.

Typical standard curve:



The curve given above is only for demonstration. It must not be used for calculation of samples of the 8-hydroxy-2'-deoxyguanosine (8-OHdG) ELISA assay.

Assay Flow Chart:



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