Package Insert

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

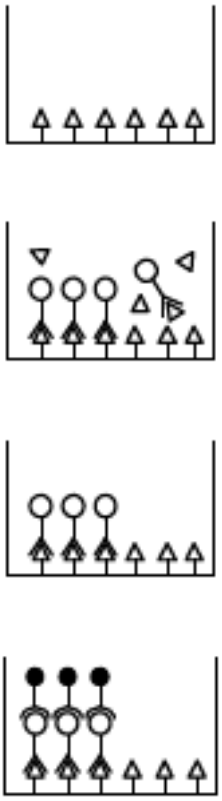
Catalog Number: HDU39-K01
96 Wells
For Research Use Only
v. 1.0

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112 Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

Intended Use:

The Eagle Biosciences 8-hydroxy-2'-deoxyguanosine (8-OHdG) Ultrasensitive 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) Ultrasensitive ELISA assay kit is for research use only and not to be used in diagnostic procedures.

Principle of Procedure:



- The 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.
- 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.
- 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.
- Unbound enzyme-labeled secondary antibody is removed by a wash step.
- Addition of a chromatic substrate results in the development of color in proportion to the amount of antibody bound to the plate.
- The color reaction is terminated and the absorbance 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: 1 vial
3. Primary Antibody Solution, Phosphate buffered saline: 1 vial (6mL)
4. Secondary Antibody, HRP-conjugated 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 (0.125, 0.25, 0.5, 1, 4, 10 ng/ml): 1 vial each
11. Plate Seal: 2 sheets

- All reagents of the 8-hydroxy-2'-deoxyguanosine (8-OHdG) Ultrasensitive ELISA assay should be stored at 2-8˚C.

- The expiry date is 9 months after manufacture and noted on the side of the kit box.

- Dilute Washing solution by 5 times (v/v) with distilled water for use.

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.
- Microtiter plate reader (measuring wavelength 450 nm).

Sample Pretreatment

To assay properly with the 8-hydroxy-2'-deoxyguanosine (8-OHdG) Ultrasensitive 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 to room temperature before beginning 8-hydroxy-2'-deoxy-guanosine (8-OHdG) Ultrasensitive 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.

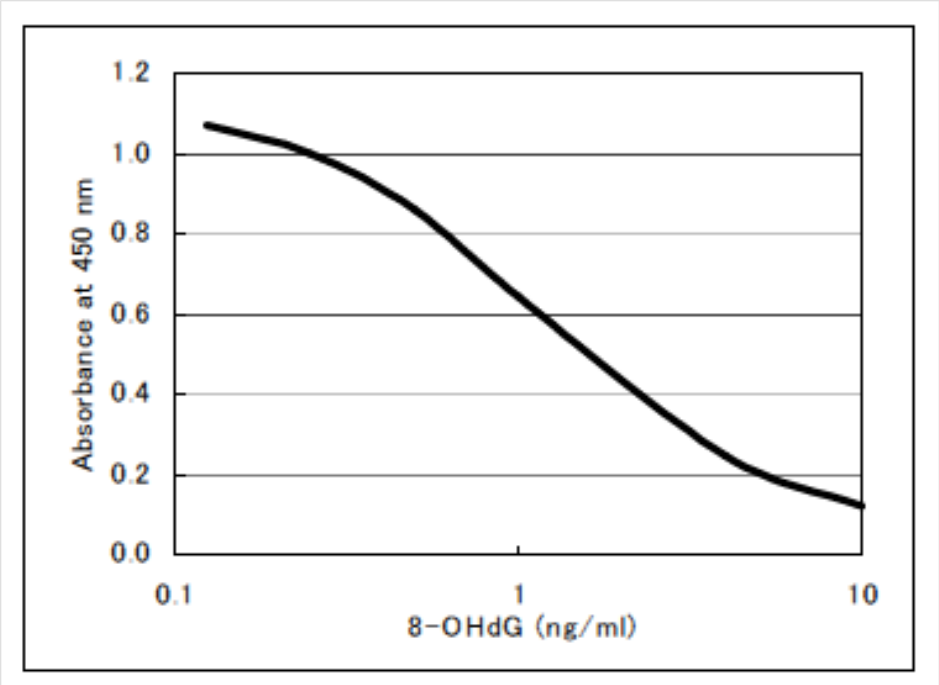
A. Reconstitute the primary antibody with the primary antibody solution. Allow dissolving completely.
B. Add 50µl of sample or standard per well.
C. Add 50µl of reconstituted primary antibody per well. Shake the plate from side to side and mix fully. Cover plate with adhesive strip, making sure it is sealed tightly. Incubate at 4℃ for overnight.
D. 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.
E. Reconstitute the secondary antibody with the secondary antibody solution. Allow dissolving completely.
F. 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.
G. At the end of the incubation period, repeat wash as in step D.
H. 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.
I. Add 100µl of the reaction terminating solution. Shake the plate from side to side and mix fully.
J. After terminating the reaction, read the absorbance at 450 nm.
K. 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.
    - 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:

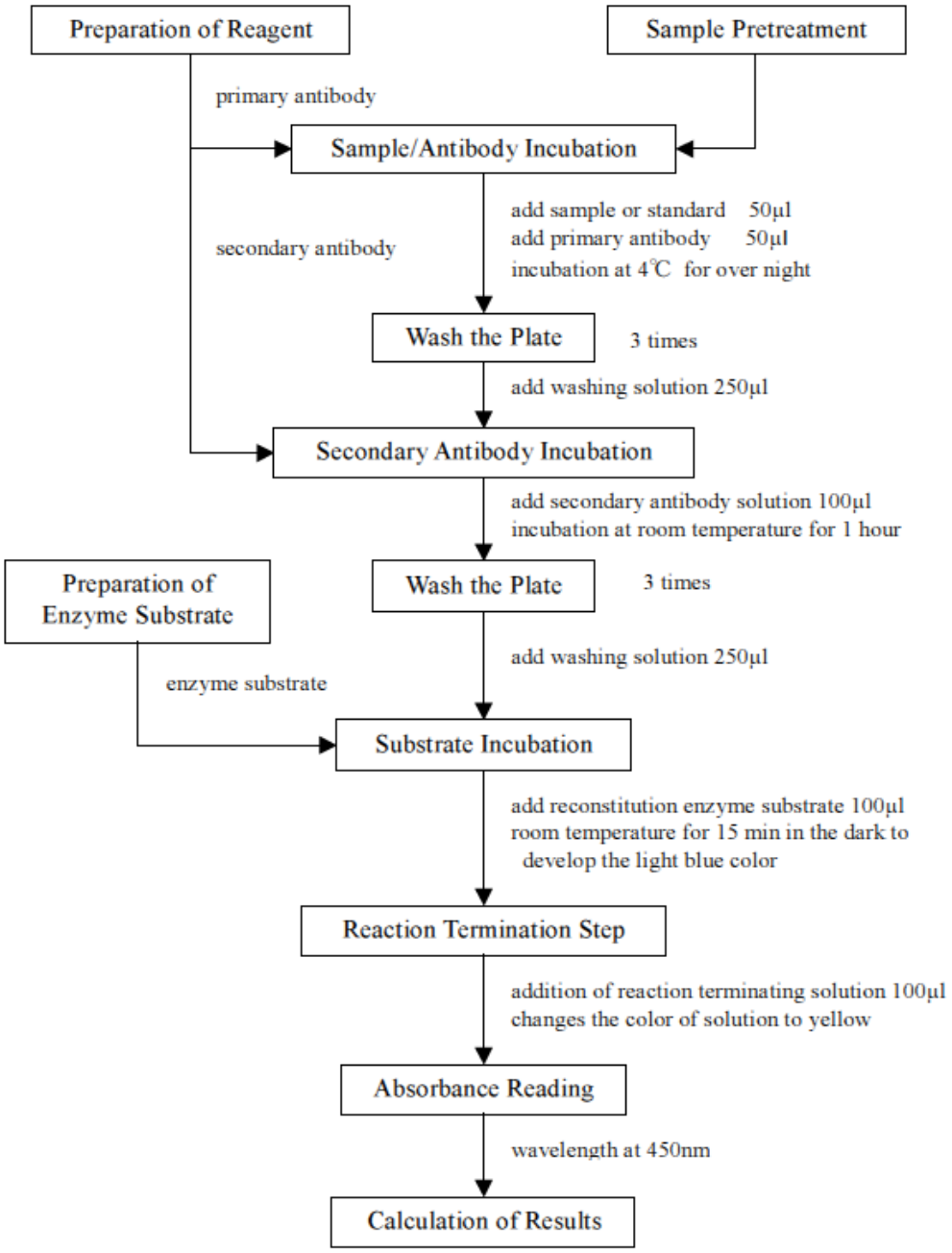
Generate the standard curve to determine the amount of 8-OHdG present in test samples. Generate the standard curve by plotting absorbance vs. log (concentration of standards). Then use the absorbance values obtained for the test samples to determine the concentrations.

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) Ultrasensitive ELISA assay.

Assay Flow Chart:



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